Volume 50 MAY, 1952 Number 5

ENDOCRINOLOGY

UNIVERSITY OF MICHIGAN
JUN 23 1952
HOSPITAL LIBRARY

BEYER, ROBERT E.
The Effect of Thyroxin Upon the General Metabolism of the Intact Chick Embryo 497

SCHILLER, SARA, EARL P. BENDITT AND ALBERT DORFMAN
Effect of Testosterone and Cortisone on the Hexosamine Content and Metachromasia of Chick Combs 504

WEEKS, M. H., J. KATZ AND N. G. FARNHAM
Organic and Inorganic Iodine Levels in Serum of Sheep Receiving Daily Oral Doses of I^{131} 511

GAUNT, ROBERT, JAMES H. LEATHEM, CONSTANCE HOWELL AND NANCY ANTONCHAK
Some Biological Properties of Different Esters of Desoxycorticosterone 521

WEXLER, BERNARD C.
Adrenal Ascorbic Acid Changes in the Gonadectomized Golden Hamster Following a Single Injection of Diethylstilbestrol 531

WYNGAARDEN, JAMES B., BENJAMIN M. WRIGHT AND PETER WAYS
The Effect of Certain Anions Upon the Accumulation and Retention of Iodide by the Thyroid Gland 537

BOTKIN, A. L., E. H. PRAYTOR, M. E. AUSTING AND H. JENSEN
Thyroid Response to Total Body X-Irradiation 550

ZARROW, M. X. AND J. T. BALDINI
Failure of Adrenocorticotropin and Various Stimuli to Deplete the Ascorbic Acid Content of the Adrenal Gland of the Quail 555

RATHER, L. J.
Constrictive Renal Hypertension in Rats Having Adrenal Autotransplants with Hepatic Portal Drainage 562

ROBERTSON, THEODORE
A Sex Difference in the Tolerance of Wistar Rats for Potassium 569

TALALAY, PAUL, MARIE MOLLOMO DOBSON, CARL M. EBERSOLE AND CHARLES HUGGINS
Physiological Interaction of Cortisone Acetate and Diethylstilbestrol. Lack of Androgenic or Estrogenic Effects of Cortisone Acetate 574

NOTES AND COMMENTS

ROTHCHILD, IRVING AND RICHARD L. RAPPORT
The Thermogenic Effect of Progesterone and Its Relation to Thyroid Function 580

ROTHCHILD, IRVING
A Simple Method for the Preparation of Aqueous Solutions of Steroid Hormones for Intravenous Administration 583

SCOTT, J. KIMBALL
The Effect of Nor-Epinephrine on the Eosinophil Count in the Mouse 585

ISSUED MONTHLY FOR THE ENDOCRINE SOCIETY
BY CHARLES C THOMAS, PUBLISHER,
SPRINGFIELD, ILLINOIS

Annual Subscription $12.00 Single Copies $1.50

asthma control with limited side-action

Orthoxine Hydrochloride, an antispasmodic and bronchodilator, was developed by Upjohn research chemists by modifying the configuration of a sympathomimetic amine molecule.

Such molecular structural change limits the action of Orthoxine mainly to bronchodilatation, thereby minimizing side-actions (vasopressor and psychomotor stimulation).

For more air, with less trouble, in controlling asthma.

℞ Orthoxine*

HYDROCHLORIDE

Bottles of 100 and 500 tablets

Orthoxine Hydrochloride (100 mg.) Tablets contain beta-(ortho-methoxyphenyl)-isopropylmethylamine hydrochloride — a bronchodilator and antispasmodic.

** Trademark, Reg. U. S. Pat. Off.*

a product of

Upjohn

Research *for medicine . . . produced with care . . . designed for health*

ENDOCRINOLOGY

VOLUME 50 MAY, 1952 NUMBER 5

Copyright 1952 by the Endocrine Society

THE EFFECT OF THYROXIN UPON THE GENERAL METABOLISM OF THE INTACT CHICK EMBRYO

ROBERT E. BEYER[1]

Department of Zoology, University of Connecticut, Storrs, Connecticut

INTRODUCTION

MURRAY (1927) demonstrated a decrease in metabolic rate per gram of body weight with age in the normal chick embryo. The only attempt to alter the metabolic rate by the administration of thyroxin and to measure it manometrically is that of Hanan (1928). Using doses ranging from 0.033 to 0.025 microgram, Hanan detected a rise in the carbon dioxide production beginning at the seventh day. The weight accretion curve was not altered.

This paper will report the effect of thyroxin injected prior to incubation on growth, differentiation and metabolic rate of the chick during the incubation period.

EXPERIMENTAL PROCEDURE

The manometric respirometer used in this study was of the constant-pressure differential type employed by Romanoff (1941). In principle it is similar to that described by Dixon (1934) and originally suggested by Adam (1920).

The eggs used in this work were of the Barred Columbian strain from the University of Connecticut poultry farm. They were collected in the morning from a single pen of an inbred strain and placed immediately in a cold room maintained at 10° C., physiological zero for chicken eggs at the preincubation stage. All eggs were incubated in a forced draft incubator at $38.0 \pm 0.5°$ C. and $55 \pm 5\%$ humidity, turned at least twice daily, and candled immediately before respiratory measurements were made.

All solutions of thyroxin employed were made from "Thyroxin Crystals" purchased from E. R. Squibb and Sons.

Wet and dry weights of both control and experimental embryos were obtained in the following manner. Embryos from the fourth to the fifth day were removed from the yolk in warm avian saline or Locke's solution, and the membranes and adhering

Received for publication January 14, 1952.

[1] Present address: Arnold Biological Laboratory, Biology Department, Brown University, Providence, R. I.

yolk granules were carefully removed. The embryo was then gently washed in a clean portion of Locke's solution, rubbed across the surface of a clean glass plate to remove excess liquid adhering to the surface of the animal, and then weighed to the nearest 0.1 mg. The four to five day old embryos were then placed in a vacuum oven intact and desiccated to constant weight (± 0.0005 gm. on two successive days). Specimens older than five days were simply opened in warm Locke's, quickly removed from their embryonic membranes, and placed in the weighing containers in an effort to lose as little blood as possible. These were then weighed intact and cut into small pieces before placing them into the vacuum oven. Constant weight was obtained in two to five days depending on the age and size of the embryo.

RESULTS

Preliminary tests: Groups of 24 eggs were injected prior to incubation with 0.25 cc. of one of the solutions shown in Table 1 and were opened on the fifth day to determine the effect of the particular concentration on the viability of the embryo. Controls consisted of non-treated animals and

TABLE 1. EFFECT OF VARIOUS CONCENTRATIONS OF THYROXIN ON EMBRYONIC MORTALITY

Micrograms injected	No. eggs incubated	No. alive at 5 days
2.5	24	0
0.25	24	5
0.025	24	17
0.0025	24	22
Water control	24	22
Untreated control	24	21

animals injected with 0.25 cc. distilled water. The results of these tests are shown in Table 1.

Since no significant difference could be noted in the two control groups all subsequent control groups consisted of non-treated animals. It may be seen from Table 1 that only the two lower concentrations of the thyroxin solutions were effectively tolerated by the embryos; hence all eggs discussed in the experiments below received 0.25 cc. of a solution containing 0.025 microgram of thyroxin immediately prior to incubation.

Oxygen uptake measurements: Table 2 contains figures showing the mean oxygen uptake per hour for groups of both the control and injected series and the numbers of animals used in a group each day. Although there was a small amount of overlapping in the maximum of the control and the minimum of the experimental groups, the means of all of these experimental groups may be seen to be nevertheless consistently higher for the experimental groups. It is evident from the decrease in the incubation period of from one to one and a half days, as well as the apparent increase in oxygen consumption of the injected embryos over the control embryos, that the thyroxin is in some way affecting a speed-up in one or more metabolic pathways. The effect of the thyroxin in augmenting total oxygen con-

sumption may be more clearly seen in Table 2 where the means of the oxygen consumption per hour per embryo are charted against the time of incubation in days. This phenomenon is discussed below.

Wet and dry weight data: An analysis of Table 2 reveals the increase in weight of the treated embryos over the control animals. On the fifth day of development the wet weight of the experimental animals is 1.6 times that of the control animals. On the sixth day this relationship has attained a magnitude of 1.8, diminishing in relative value from this point until the time of hatching. A similar increase is observed in the dry weight data

TABLE 2. OXYGEN CONSUMPTION, WEIGHTS AND METABOLIC RATE OF CONTROL AND INJECTED ANIMALS

Day of inc.	Control		Injected		Control			Injected			Control	Injected
	No. used	Mean O_2 uptake/hr., cc.	No. used	Mean O_2 uptake/hr., cc.	No. used	Mean dry wt., gm.	Mean wet wt., gm.	No. used	Mean dry wt., gm.	Mean wet wt., gm.	Metabolic rate[1]	Metabolic rate[1]
1	3	0.13	6	0.42								
2	3	0.90	5	0.42								
3	3	0.15	4	0.53								
4	3	0.40	6	0.68	6	0.0022	0.0834	6	0.0030	0.0896	4.77	7.05
5	4	0.44	6	0.93	3	0.0052	0.0841	5	0.0081	0.1317	5.23	7.06
6	3	0.75	6	1.35	3	0.0121	0.2201	3	0.0222	0.4022	3.41	3.36
7	5	2.45	6	2.75	3	0.0237	0.4245	3	0.0371	0.6069	5.77	4.53
8	6	2.64	6	3.69	3	0.0446	0.7822	3	0.0576	1.0007	3.37	3.67
9	6	3.83	6	5.79	3	0.0796	1.2130	3	0.1073	1.5534	3.16	3.73
10	7	5.18	6	6.81	3	0.0927	1.3964	3	0.1753	2.5122	3.71	2.71
11	7	7.49	6	9.59	3	0.1422	2.0901	3	0.2623	3.3843	3.58	2.83
12	5	8.95	6	13.00	3	0.2315	3.1437	3	0.3972	4.7306	2.85	2.75
13	6	11.62	6	16.56	3	0.4161	4.9981	3	0.5928	6.6475	2.32	2.49
14	6	12.70	6	20.03	3	0.5911	6.7239	2	0.7018	8.8278	1.89	2.27
15	5	17.14	3	23.29	3	0.6219	7.0187	3	1.2404	10.2468	2.44	2.27
16	6	16.37	3	29.37	2	2.0571	13.4842	2	2.8592	16.1078	1.21	1.82
17	6	18.42	6	25.98	2	2.6263	15.1138	2	3.6095	19.4746	1.22	1.33
18	6	17.95	6	26.21	2	4.1944	21.3156	2	4.7553	24.5143	0.84	1.07
19	6	20.58	5	27.00	2	3.9715	21.8517	2	4.9078	27.6314	0.94	0.98
20	5	20.78	4	48.40	2	5.2188	22.9820	2	4.7124	25.6842	0.90	1.88
21	5	43.81	—	—	2	6.2247	26.0360	—	—	—	1.68	—

[1] Calculated as oxygen uptake in cc./hr./gm. wet weight.

(Table 2). Contrast between size of control and treated embryos is shown photographically in plate 1.

The weight data in Table 2 are of intrinsic value with respect to growth acceleration and indication of attainment of a larger hatching weight and an earlier hatching date (one to one and one half days); moreover, they will serve as standards of reference for computation of oxygen consumption relative to a fixed protoplasmic value.

Oxygen consumption data and metabolic rate calculations: Byerly (1932) reported the proportion of the weight of the extraembryonic membranes in regard to the total living matter in the chicken's egg at progressive stages of development. His study showed that the yolk sac grows much more rapidly than the embryo proper during the first week of development, but grows at approximately the same rate for the last two weeks. Allantoic growth beginning on the fourth day continues very vigorously until nine and one half days of development have passed, after which the

allantoic weight in per cent of the total weight declines.

Until consideration of the true metabolic rate of the chick embryo by Needham (1932), metabolism of the extraembryonic membranes had been neglected (Bohr and Hasselbalch, 1900; Atwood and Weakley, 1924; Lamson and Edmond, 1914; Murray, 1926, 1927; and Hanan, 1928). It seems logical that the yolk sac with its layer of entoderm and its highly vascular splanchnic mesoderm must possess an active oxidative metabo-

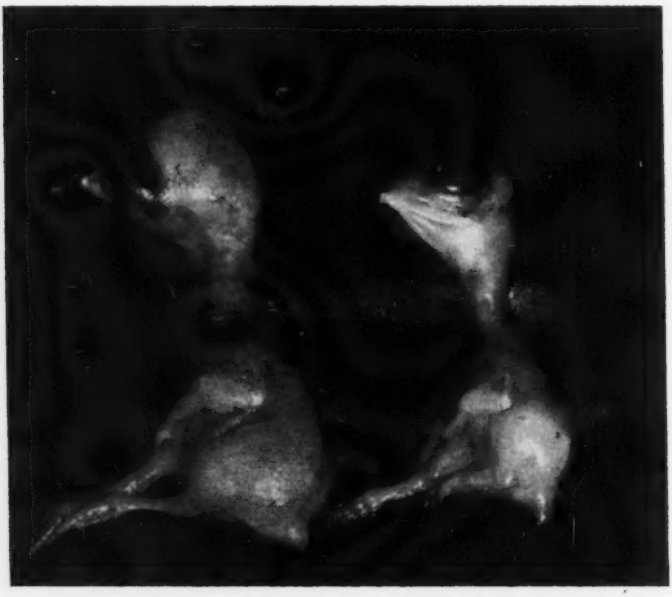

PLATE 1. Photograph of control (right) and experimental (left) chick embryos at thirteen days of development.

lism of its own, and the allantois must also have an intrinsic respiration. Consequently, it must be kept in mind that the figures given here for the oxygen consumption are those figures derived from the intact egg, the sum total of all of the oxidative processes occurring in the egg at the time of measurement. Therefore, the data for "metabolic rate" in Table 2 will at once be recognized as spuriously high; these figures, based on total oxygen consumption but referred to embryonic substance only, are for comparative purposes only. Except in the instance of a selective action of thyroxin on one or all of the extraembryonic membranes, the comparison in this report is valid.

Comparison of the figures given in Table 2 for the metabolic rate calcu-

lated as the oxygen consumption of the embryo in cc./hr./gm. wet weight for experimental and control groups reveals that no significant difference exists between the metabolism of the treated an tahe untreated embryos at any time during development. It may be said at this point that the injection of thyroxin into the yolk of preincubated chicken eggs has not significantly altered the metabolic rate of the embryo during the successive stages of its embryonic development, even though evidence for accelerated growth and/or differentiation changes are presented.

It is also noteworthy that the observations of the writer confirm those of Kuo and Shen (1937) and Windle and Barcroft (1938) that the chick embryo, during the nineteenth day of development, begins rhythmic respiratory movements which increase in magnitude and regularity until the time of hatching when the complete oxygen supply of the embryo is gained by means of pulmonary activity.

DISCUSSION

Response of adult organisms to thyroxin by increasing metabolic rate is well established, though the precise role of thyroxin in accomplishing this effect is not clear. The problem is doubly confusing in embryonic tissues whose responsive capacities may be different from those of the adult or, particularly at early stages, entirely absent.

The chick thyroid has been shown (Bradway, 1929; Sun, 1932; and Hopkins, 1935) by means of morphological and cytochemical tests to commence thyroxin production between the tenth and twelfth days. A more recent analysis has been made (Hansborough and Khan, 1951) by the use of radioautographs which show the initial accumulation of radioactive iodine by the chick embryonic thyroid to take place on the eleventh day. The indication of synthesis of the thyroxin molecule or at least the addition of two iodine atoms to the tyrosine molecule to form the diiodotyrosine is clear.

Willier (1924) made chorio-allantoic thyroid grafts which resulted in emaciated embryos. However, Table 2 shows that the wet weights of the treated embryos of this report have increased in marked contrast to the behavior of Willier's embryos. Simultaneously, the oxygen uptake is seen to increase, but this increase is an increase in direct proportion to the weight increase, demonstrating that the oxygen uptake per unit of wet or dry weight is unchanged by thyroxin administration.

The present data demonstrate inescapably a thyroxin induced acceleration of growth that begins at approximately day four. The parallel increase of wet and dry weights points to a uniform, systematic synthesis of organic molecules rather than increased acquisition of water alone. Increased mass therefore might be a result of increased cellular size or in-

creased mitotic rate. Neither histological nor cytological examination has been made to ascertain the actual morphological response.

The suggestion fo accelerated differentiation (plate 1), supported as well by a shortened incubation period, is clear. The classic observations of Gudernatsch (1913) on amphibian embryos may represent an exaggerated though parallel example of the same phenomena. Hoffman and Gudernatsch (1931, 1933) and Gudernatsch and Hoffman (1931), after investigating the role of a variety of amino acids, conclude that some are sufficient for maintenance of the tadpole, others are influential and perhaps required in the growth process, while others "of a structure which approached that of diiodotyrosine (and thyroxin) are of definite influence in the process of differentiation." Embryonic tissues, prior to thyroid and pituitary function, may be incapable of responding metabolically to thyroxin, but may have some capacity to respond by growth and differentiation to the tyrosine or phenylalanine portions of the thyroxin molecule.

The writer is grateful to Professor Hugh Clark for his generous guidance and encouragement during the course of this investigation and the writing of the manuscript.

SUMMARY

Eggs from a Barred Columbian stock of *Gallus domesticus* were injected with 0.025 microgram of thyroxine at the pre-incubation stage. The oxygen uptake was measured manometrically and the wet and dry weights during progressive states were obtained. From these data the metabolic rate of normal and experimental embryos was calculated.

Several conclusions are drawn from this study: An increase in both wet and dry weights of the embryo starting at at least four days of development; an increase in the oxygen uptake per embryo during all stages of development of the embryo; no significant increase in the metabolic rate of the chick embryo during any part of its embryonic development, calculated on the basis of either wet or dry weights; a proportional increase of weight and oxygen uptake resulting in a metabolic rate equivalent to that of the control animals; a decrease in the incubation period of from one to one and one half days.

REFERENCES

ADAM, N. K.: *Biochem. J.* **14**: 679–685. 1920.
ATWOOD, H. AND C. E. WEAKLEY, JR.: *West Virg. Agric. Exp. Sta. Bull.* **185**: 1924.
BOHR, C. AND H. A. HASSELBALCH: 1900 (Cited by Needham, 1931).
BRADWAY, W.: *Anat. Rec.* **42**: 157–167. 1929.
BYERLY, T. C.: Unpublished. 1932 (Cited by Needham, 1932).
DIXON, M.: *Manometric Methods*, London, Cambridge Univ. Press. 1934.

GUDERNATSCH, J. F.: *Archiv f. Entwicklungsmechanik* **35:** 457–483. 1913.
GUDERNATSCH, J. F. AND O. HOFFMAN: *Am. J. Physiol.* **97:** 527–528. 1931.
HANAN, E. B.: *Proc. Soc. Exp. Biol. and Med.* **24:** 422–425. 1928.
HANSBOROUGH, L. A. AND M. KHAN: *J. Exp. Zool.* **116:** 447–453. 1951.
HOFFMAN, O., AND J. F. GUDERNATSCH: *Proc. Soc. Exp. Biol. and Med.* **28:** 731–732. 1931.
HOFFMAN, O., AND J. F. GUDERNATSCH: *Endocrinology* **17:** 240–249. 1933.
HOPKINS, M. L.: *J. Morph.* **58:** 583–614. 1935.
KUO, Z. Y. AND T. C. R. SHEN: *J. Comp. Psychol.* **24:** 49–58. 1937.
LAMSON, G. H., JR. AND H. D. EDMOND: *Storrs Agric. Exp. Sta. Bull.* **76:** 1914.
MURRAY, H. A., JR.: *J. Gen. Physiol.* **9:** 603–619. 1925–26.
MURRAY, H. A., JR.: *J. Gen. Physiol.* **10:** 337–343. 1927.
NEEDHAM, J.: *Chemical Embryology,* Cambridge Univ. Press. 1931.
NEEDHAM, J.: *Proc. Roy. Soc. Lond.,* B. **112:** 98–113. 1932.
ROMANOFF, A. L.: *J. Cell. and Comp. Physiol.* **18:** 199–214. 1941.
SUN, T. P.: *Physiol. Zool.* **5:** 384–396. 1932.
WILLIER, B. H.: *Am. J. Anat.* **33:** 67–103. 1924.
WINDLE, W. F. AND J. BARCROFT: *J. Am. Physiol.* **121:** 684–691. 1938.

EFFECT OF TESTOSTERONE AND CORTISONE ON THE HEXOSAMINE CONTENT AND METACHROMASIA OF CHICK COMBS[1]

SARA SCHILLER, EARL P. BENDITT
AND ALBERT DORFMAN

From La Rabida Jackson Park Sanitarium and the Departments of Pathology and Pediatrics, The University of Chicago, Chicago, Illinois

IN 1949, Boas reported the isolation of hyaluronic acid from the cock's comb. It was suggested that hyaluronic acid comprises a considerable proportion of the mucopolysaccharide component of the comb (Ludwig and Boas, 1950) and that its deposition in the cock's comb depends on androgen stimulation.

The present investigation was designed to study the effect of testosterone and 17-hydroxy-11-dehydrocorticosterone (Kendall's Cpd. E) on the mucopolysaccharide content of the chick comb. For the determination of hexosamine, a method which does not distinguish between glucosamine and galactosamine was used.

MATERIALS AND METHODS

One day old White Leghorn male chicks were placed in thermostatically controlled battery brooders and fed chick starter mash and water. The chicks were three days old at the beginning of the experiment. Testosterone propionate (Schering Corporatoin) was made up to contain the total dose in 0.35 ml. of corn oil and 0.05 ml. was applied locally to the comb once daily for seven days. In the intiail experiments (Table 1), crystalline cortisone acetate was dissolved in corn oil and applied to the chick's comb in the same volume and manner as the testosterone. In all other experiments, 0.1 ml. of a suspension,* containing 2.5 mg. of cortisone acetate, was administered intramuscularly each day for seven days. The animals used for control received the appropriate vehicle. Twenty-four hours after the last hormone injection the chicks were sacrificed. The combs were removed, as described by Frank *et al.* (1942), and weighed on a Roller-Smith torsion balance.

A number of combs from each treatment group were dried to constant weight and the hexosamine content of the dry comb, after hydrolysis, was determined without preliminary extraction. The remaining combs from each treatment group, 6–10 combs per group, were combined in order to obtain sufficient material for extraction and analysis. They were minced and placed in acetone (6 ml. per comb). The method of Boas (1949) for extracting hyaluronic acid from combs was used. After defatting with

---

Received for publication January 18, 1952.

[1] This investigation was supported by a grant from the Chicago Heart Association.

* The cortisone acetate was (cortone) supplied through the courtesy of Merck and Co.

acetone, the combs were homogenized in a glass homogenizer and extracted five times with a 5% solution of sodium acetate. The sodium acetate extracts were combined and hyaluronic acid precipitated by the addition of 1.5 volumes of ethyl alcohol. After standing at 4° overnight, the supernatant was removed by centrifugation and the precipitate redissolved in 5% sodium acetate solution and was made up to a volume of 5 or 10 ml., depending upon the quantity of precipitate. Aliquots of the solution were hydrolyzed with 4*N* hydrochloric acid in sealed tubes immersed for 8 hours in a boiling water bath. Hexosamine was measured in the hydrolysate by a modification of the method of Schloss (1948). One ml. of the hydrolysate, containing 10–150 μg of hexosamine, is diluted to 5 ml. with water. The diluted material is shaken with 10 mg. of active carbon (Darco G-60) and filtered through Whatman No. 1 filter paper. One ml. of the clear supernatant is transferred to a 10 ml. volumetric tube and neutralized with 4*N* KOH. After adding 1.0 ml. of acetylacetone reagent (made up to contain 2.0 ml. of acetylacetone, 1.76 ml. of conc. HCl and 5.3 gm. of Na_2CO_3 in 40 ml. of water) and water to make a total volume of 3 ml., the tubes are immersed in a boiling water bath for 30 minutes. During the entire boiling period the level of water in the bath is maintained ½ inch above that in the tubes. After the thirty minutes boiling period the tubes are cooled to room temperature. Absolute ethanol is added to bring the volume to 8 ml. and the color is developed by the addition of 1.0 ml. of Ehrlich's reagent. Ehrlich's reagent was made up to contain 800 mg. of recrystallized p-dimethylaminobenzaldehyde (Eastman-Kodak) dissolved in 30 ml. of absolute ethanol and diluted to 60 ml. with concentrated HCl. The volume in the tube is brought to 10 ml. with absolute alcohol, the contents mixed and transferred to a colorimeter tube. Optical density is measured at 535 μg exactly 30 minutes after the addition of Ehrlich's reagent. All determinations were carried out in duplicate. The concentration was calculated from the optical density value by reading from a curve drawn up for 2–30 μg of glucosamine using crystalline glucosamine hydrochloride as standard. With this method of hydrolysis and measurement, 91–97% recovery of crystalline glucosamine hydrochloride was obtained consistently.

Sections of combs were frozen-dried or fixed by a 4% solution of formaldehyde in 80% alcohol. The combs were embedded in pure paraffin (m.p. 57° C.) and 6 micra sections were cut. The frozen-dried sections were mounted without contact with water by finger pressure and warming and the paraffin was removed with petroleum ether. Post-fixation treatment of the frozen-dried material varied with the subsequent staining procedures. Prior to hematoxylin-eosin or van Gieson's stain, the sections were immersed in neutral 4% formaldehyde for approximately one hour. Toluidine blue staining was preceded by fixation in 95% alcohol for 16 hours or in a mixture of equal parts of freshly filtered 8% lead subacetate and 16% formaldehyde for one hour (Glick, 1949). Overnight fixation in 95% alcohol was used prior to periodic acid-Schiff stain.

RESULTS

In the initial experiments, a total of 10–1000 μg. of cortisone acetate applied to the chick's comb over a period of seven days resulted in no consistent effect on the hexosamine content of the extracted polysaccharide or the dry comb. These results are presented in Table 1. The hexosamine content of combs from chicks treated with 160 /g. of testosterone propionate, however, showed a 3–4 fold increase over that of the controls. This increase was not affected by the simultaneous application of cortisone acetate. The

higher level of hexosamine in the comb of testosterone treated chicks appears to be, in part, associated with the stimulated growth of these combs. That more hexosamine accumulates following testosterone administration than is accounted for by the increased size of the comb is evident from an increase in the concentration (mg. of hexosamine per gram of dry tissue).

TABLE 1. EFFECT OF LOCAL APPLICATION OF TESTOSTERONE PROPIONATE AND CORTISONE ACETATE UPON THE HEXOSAMINE CONTENT OF THE CHICK'S COMB

Treatment		Hexosamine content			
Testosterone propionate	Cortisone acetate	Extract[1]		Dry comb[2]	
μg.	μg.	μg. per gram	μg. per comb	mg. per gram	μg. per comb
0	0	440	21	8.2	47
0	10	460	19	6.8	42
0	100	210	10	8.1	35
0	1000	530	20	7.6	45
160	0	520	68	10.6	163
160	10	690	72	10.5	120
160	100	640	60	10.6	103
160	1000	760	77	11.3	130

[1] Values obtained from a pool of 7–10 combs for each treatment group.
[2] Values obtained from a pool of 2 combs for each treatment group.

Since local application of cortisone acetate appeared to have no effect on the hexosamine content of the chick's comb, two experiments were performed in which a suspension of this adrenal steroid was administered intramuscularly. As in the case of local application, no demonstrable effect on the hexosamine content of the comb was observed following intramuscular injections of 2.5 mg. of cortisone acetate per day for seven days (Table 2).

The increase in the hexosamine content of the comb in response to the application of 320 μg. of testosterone propionate was similar to that obtained with 160 μg. and was not influenced by simultaneous injections of cortisone. Of interest, is the fact that there were no differences in the comb weights or in the comb wt. (mg.)/body wt. (gm.) ratios between the chicks treated with 160 μg. and 320 μg. testosterone propionate.

Since testosterone propionate was administered simultaneously with cortisone acetate, the possibility existed that a response to the latter might have been masked by the great sensitivity of the chick comb to testosterone. Chick combs were pretreated, therefore, for seven days with a total dose of 160 μg. of testosterone propionate after which 2.5 mg. of cortisone acetate per day was administered intramuscularly for seven days. Since the chicks were 10 days old at the start of cortisone treatment and 18 days of age when sacrificed, the possibility of an endogenous source of testosterone during this period of the experiment was recognized. The data in Table 3, indicate that cortisone acetate has no effect on the concentration

TABLE 2. EFFECT OF INTRAMUSCULAR ADMINISTRATION OF CORTISONE ACETATE ON THE HEXOSAMINE CONTENT OF THE CHICK COMB TREATED SIMULTANEOUSLY WITH TESTOSTERONE PROPIONATE

Treatment		Hexosamine content					
Testosterone propionate	Cortisone acetate	Extract[1]			Dry comb[2]		
		No. of combs	µg. per gram	µg. per comb	No. of combs	mg. per gram	µg. per comb
µg.	mg.						
Experiment 1							
0	0	10	113	12	2	7.3	61
0	17.5	10	104	13	2	6.8	63
160	0	10	127	43	2	9.8	195
160	17.5	9	188	53	2	10.5	178
Experiment 2							
0	0	7	134	7	7	5.8 ± 0.49[2]	46.7 ± 6.6[2]
0	17.5	7	124	5	7	6.0 ± 0.75	57.0 ± 10.2
160	0	6	315	36	6	9.4 ± 0.65	200.8 ± 23.8
160	17.5	6	247	26	6	10.0 ± 0.40	192.3 ± 38.0
320	0	6	248	40	6	10.1 ± 1.03	191.5 ± 27.0
320	17.5	6	327	53	6	10.4 ± 0.37	266.7 ± 31.2

[1] Values obtained from a pool of the combs except in experiment 2 where the hexosamine was determined for each dry comb.
[2] Means and standard error.

of hexosamine. The hexosamine content of the combs from the cortisone treated chicks which had been pretreated with testosterone, however, was decreased. This depression in the hexosamine content was associated with an inhibition in the rate of comb growth as compared with that of chicks which had received only testosterone.

Considerably higher levels of hexosamine were found in unextracted dried combs as compared with comb extracts. It is not unlikely that the hexoasmine in the dry comb represents more than the glucosamine of hyaluronic acid. In order to ascertain where in the extraction procedure the

TABLE 3. EFFECT OF INTRAMUSCULAR ADMINISTRATION OF CORTISONE ACETATE ON THE HEXOSAMINE CONTENT OF THE CHICK COMB PRETREATED WITH TESTOSTERONE PROPIONATE

Treatment		Comb weight[1]	Hexosamine content[2]	
Testosterone propionate (applied the first week)	Cortisone acetate (injected the second week)			
µg.	mg.	mg.	µg./gram	µg./comb
0	0	225.7 ± 82.6 (6)	597	125
0	17.5	296.2 ± 62.7 (5)	464	103
160	0	597.8 ± 134.8 (6)	844	490
160	17.5	358.8 ± 126.3 (5)	842	225

[1] Means and standard deviation. Figures in parenthesis indicate the number of combs.
[2] Determinations obtained from sodium acetate extracts of a pool of the combs for each treatment group.

loss of the total hexosamine occurred, a single comb of a two month old cock was defatted with acetone and divided into three portions. One aliquot of comb was dried, a second aliquot was extracted repeatedly with a 5% solution of sodium acetate, while the combined sodium acetate extracts of a third aliquot were precipitated with 1.5 volumes of 95% ethyl alcohol. The hexosamine values were 27.1 mg. in the dried comb, 12.3 mg. in the sodium acetate extract and 6.3 mg. in the alcohol precipitate. It is obvious that all of the hexosamine-containing substances are not extracted from the tissue with dilute sodium acetate and that only a portion of the soluble material is precipitated.

The histologic observations on the chick combs treated with testosterone were essentially similar to those of Ludwig and Boas (1950). In the middle layer of loose connective tissue, the stimulated combs showed fibroblast and capillary proliferation, with production of fine collagen fibers and the appearance of substantial amounts of metachromatic material. The metachromatic substance appeared in the intercellular, interfibrillar spaces and was granular. The quantity of metachromatic material increased with the dose of testosterone administered and in general paralleled the hexosamine content of the comb. Cortisone caused no diminution in metachromasia or fibroblastic activity in combs under stimulation by testosterone. Corresponding to the quantity of metachromasia, there appeared a looseness and thinning of the collagen fibers.

DISCUSSION

The striking effect of cortisone on pathological processes involving connective tissue suggested the possibility that this substance may have a specific effect on some or all of the components of connective tissue. Studies of Ragan *et al.* (1949) and of Castor and Baker (1950) indicate that an inhibition of the formation of connective tissue may result from the action of cortisone. These investigations, however, give no clue as to the chemical nature of this inhibition. Recently, Layton (1951 a, b) reported that cortisone may interfere with the formation of chondroitin sulfate. His conclusions are drawn from *in vitro* inhibition of esterification of sulfate labeled with radioactive sulfur in embryonic and wound tissues and from a decreased concentration of bound sulfate in the skin of intact cortisone-treated rats. Since these animals excreted large quantities of sulfate the decreased concentration of fixed sulfate in the skin may have been the result of increased protein catabolism, consequent to cortisone treatment.

A large component of the cock's comb is connective tissue with a relatively high percentage of mucopolysaccharide (Boas, 1949), (Ludwig and Boas, 1950). If cortisone specifically affects the elaboration of mucopolysaccharides, synthesis in the chick comb should be inhibited. No such in-

hibition was found. No evidence of a significant reduction in the concentration of hexosamine was obtained following short-term administration of cortisone. Furthermore, the metachromatic material which appeared in sections of combs from animals treated with testosterone, was not diminished by cortisone treatment.

The exact nature of toluidine blue metachromasia is not clear, although current evidence suggests (Lille *et al.*, 1951) that the sulfated polysaccharides are largely responsible for the metachromatic staining properties of tissues. Ludwig and Boas (1950) pointed out that the metachromasia in their sections of cock's comb was only partially sensitive to treatment with streptococcal hyaluronidase, an enzyme which does not attack chondroitin sulfate. Testicular hyaluronidase, however, removed the metachromatic material entirely. Since this enzyme acts on both chondroitin sulfate and hyaluronic acid a substantial quantity of sulfated mucopolysaccharide may be present in the cock's comb. Our studies give no indication that cortisone affects the production of metachromatic sulfated mucopolysaccharides or any other hexosamine-containing substance in the chick comb.

Connective tissue in various locations is highly specific in behavior. Such specificity is well illustrated by the striking growth response of the comb to androgenic substances. The present data suggest that under the influence of testosterone the rate of mucopolysaccharide production is greater than the growth rate of the chick comb.

It would appear that caution is indicated in drawing any general conclusions regarding the effect of cortisone on the synthesis of mucopolysaccharides. The failure of cortisone to affect the hexosamine-containing and /or metachromatic mucopolysaccharides in the cock's comb may not correlate with its effects on other types of connective tissue.

SUMMARY

The effects of testosterone propionate and cortisone acetate were studied on the mucopolysaccharide of connective tissue as measured by the level of hexosamine and the production of metachromasia in chick combs.

Local application of testosterone propionate to chick combs for seven days caused a marked increase in the hexosamine-containing mucopolysaccharides of the comb. The increased level of mucopolysaccharide was associated with a stimulation of comb growth and the appearance of metachromasia. None of these responses were affected by the simultaneous administration of cortisone acetate whether applied locally or injected intramuscularly.

Cortisone acetate administered alone for seven days was without effect on the comb size or the mucopolysaccharide content. However, when administered to chicks pretreated with testosterone propionate an inhibition

in the rate of comb growth was obtained. In these combs, the concentration of hexosamine remained unchanged, but the level of hexosamine per comb was less than that from animals receiving only testosterone.

REFERENCES

Boas, N. F.: *J. Biol. Chem.* **181**: 573. 1949.

Castor, C. W. and B. L. Baker: *Endocrinology* **47**: 234. 1950.

Frank, R. T., E. Klempner, F. Hollander and B. Kriss: *Endocrinology* **31**: 63. 1942.

Glick, D.: Techniques of Histo- and Cytochemistry, Interscience Publishers, Inc., New York, 1949.

Layton, L. L.: *Proc. Soc. Exptl. Biol. and Med.* **76**: 596. 1951a.

Layton, L. L.: *Arch. Biochem. Biophys.* **32**: 224. 1951b.

Lillie, R. D., E. H. Emmart and A. M. Laskey: *Arch. Path.* **52**: 363. 1951.

Ludwig, A. W. and N. F. Boas: *Endocrinology* **46**: 291. 1950.

Ragan, C., E. L. Howes, C. M. Plotz, K. Meyer and J. W. Blunt: *Proc. Soc. Exptl. Biol. and Med.* **72**: 718. 1949.

Schloss, B.: Thesis, New York University, 1948.

ORGANIC AND INORGANIC IODINE LEVELS IN SERUM OF SHEEP RECEIVING DAILY ORAL DOSES OF I^{131} [1,2]

M. H. WEEKS, J. KATZ AND N. G. FARNHAM[3]

Biology Section, Radiological Sciences Department, General Electric Company, Richland, Washington

INTRODUCTION

THE use of I^{131} for research purposes has contributed greatly to modern concepts of thyroid physiology. The ionizing radiations produced by this isotope are being utilized therapeutically in attempts to control the hormone-producing ability of the thyroid gland. However, little conclusive information is available concerning harmful effects on the thyroid resulting from chronic administration of pharmacological amounts of I^{131}. Further information is essential to establish maximum permissible concentrations of I^{131} for persons and animals exposed over long periods of time and for determination of dosages which may be considered safe for therapeutic purposes.

To determine the pathological effects of chronic oral admnistration of I^{131}, 480 μc. of carrier-free I^{131} were fed daily to nine Suffolk ram lambs. In addition to histopathological examination of the thyroid glands at necropsy (to be reported later[4]), the organic and inorganic iodine levels in the serum of these animals were determined at intervals during the experiment, since changes from normal iodine values often serve as an indication of radiation damage to the thyroid (Feller *et al.*, 1949). External radiation monitoring of the thyroids was performed daily to measure the I^{131} concentrating ability of the glandular tissue.

Received for publication January 26, 1952.

[1] Based on work performed under Contract No. W-31-109-Eng-52 for the Atomic Energy Commission.

[2] The I^{131} used in this investigation was supplied by the Isotopes Division, United States Atomic Energy Commission.

[3] Present address: Physical Sciences Department, University of Idaho, Moscow, Idaho.

[4] The work reported here is part of a program of research on the toxicity of I^{131} on sheep which is being conducted in this laboratory.

METHODS

I^{131} Feeding Schedule

The 9 experimental sheep (approximately 8 months old at the start of the experiment) were divided into 3 groups of 3 sheep each. Animals in Group I were fed daily, in addition to their normal diet, one drilled food pellet containing 480 μc. of I^{131}. When the radioactivity in the thyroid, as determined by external monitoring, reached a peak and began to fall, indicating a loss in ability to concentrate iodine, the animals were sacrificed, and damage to the thyroid gland was confirmed by histological studies.

The sheep in Group II were similarly fed 480 μc. I^{131} per day until a decrease in radioactivity of the thyroid was observed. I^{131} feedings were then discontinued and several weeks later these animals were fed a tracer dose of I^{131} (60–72 μc.) in order to evaluate the regenerative power of thyroid tissue.

Sheep in Group III were fed 480 μc. of I^{131} daily throughout the entire experiment in spite of a continuous decrease in radioactivity levels as detected by external monitoring.

Analytical Methods

The total protein-bound iodine, the protein-bound I^{131}, and the inorganic I^{131} levels in the serum of all experimental animals were simultaneously determined at frequent intervals by the following procedures:

Total protein-bound iodine

The methods employed for precipitating and washing the proteins, ashing the precipitates, and dissolving the iodides from the ash were those described by Barker and Humphrey (1950). The colorimetric determination of the iodide was performed as described by these workers, except that in our experience it was found necessary to prepare a standard curve daily with every series of unknowns. This curve was prepared by placing 8.0 ml. of doubly distilled water, 1.0 ml. of 10 per cent zinc sulfate, and 1.0 ml. of 0.5 N sodium hydroxide in a series of tubes and carrying out the entire analytical procedure including ashing the precipitates and dissolving the ash. To 3.0 ml. aliquots of these solutions were added known amounts of potassium iodide, 0.5 ml. of 0.1 N sodium arsenite, and doubly distilled water to make 10.0 ml. These standards were incubated in a water bath at 36.0° C. $\pm$0.1 simultaneously with the tubes containing the unknown samples. The degree of decolorization after addition of ceric sulfate was measured with a Coleman Junior Spectrophotometer previously adjusted to 100 per cent light transmission through distilled water at 420 microns. When the per cent transmission was plotted against the concentration of potassium iodide on semi-log paper, the standard graph was a relatively straight line.

Protein-bound I^{131}

The proteins in 1.0 ml. of serum were precipitated by the addition of 1.0 ml. of 10 per cent zinc sulfate solution and 1.0 ml. of 0.5 N sodium hydroxide. The mixture was centrifuged and the supernatant saved for analysis of inorganic I^{131}. The precipitate was washed three times with 10 ml. portions of doubly distilled water and the washings were combined with the original supernatant. The washed precipitate was suspended in one ml. of 4 N sodium carbonate and the suspension dried in an oven at 90–95° C. for 12 to 18 hours. The residue was ashed in a muffle furnace for three hours at 600° C. After cooling, 1.0 ml. of 2.5 per cent potassium iodide carrier solution was added and the ash dissolved in 10 to 15 ml. of 0.005 N sodium bisulfite and 1.0 ml. of 4 N nitric acid.

When bubbling ceased, the solution was further acidified with a few drops of concentrated nitric acid and transferred to a centrifuge cone. One ml. of 3.0 per cent silver nitrate solution was added, the precipitated silver iodide separated by centrifugation and transferred, by using several ml. of 95 per cent ethyl alcohol, to a one inch stainless steel counting plate (Palmer, 1951).

Inorganic I^{131}

One ml. of 2.5 per cent potassium iodide solution was added to the combined supernatant and washings obtained as described above. After acidifying with 4 drops of 4 N nitric acid, 1.0 ml. of a 3.0 per cent silver nitrate solution was added and the precipitate allowed to settle for several minutes. After centrifuging, the supernatant was discarded and the precipitate transferred to a one inch stainless steel counting plate by using a small volume of 95 per cent ethyl alcohol.

Plated silver iodide samples were counted, using a thin mica window G.M. tube. All counting rates were assigned 90 per cent confidence limits (Healy *et al.*, 1950). Disintegrations per minute were calculated from the corrected counts per minute and converted to micrograms of I^{131} per ml. of serum.

Total I^{131} levels per ml. of serum were calculated by adding the fractions contributed by the protein-bound I^{131} and the inorganic I^{131}. Results agreed within 5 to 10 per cent with values obtained by assay of total I^{131} by plating and counting the total radioactivity in 1 ml. of serum.

RESULTS AND DISCUSSION

Figure 1 represents the levels of total protein-bound iodine and protein-

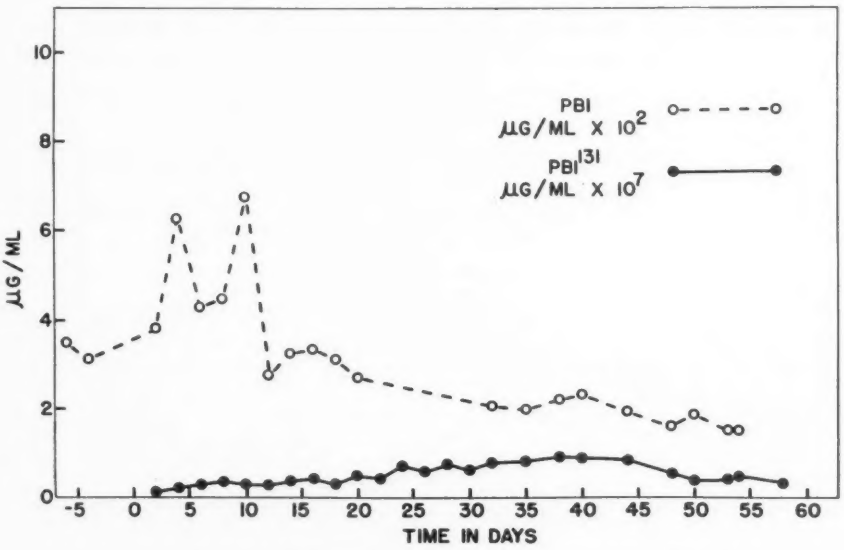

FIG. 1. Serum protein bound iodine in sheep receiving daily oral doses of 480 μc. of I^{131}. Sheep No. 1, Group I.

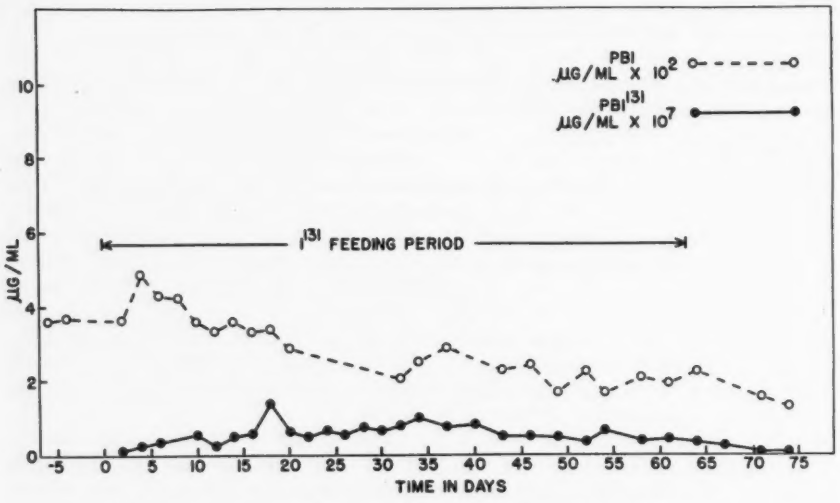

FIG. 2. Serum protein bound iodine in sheep receiving daily oral doses of 480 μc. of I^{131}, Sheep No. 4, Group II.

bound I^{131} in the serum of a typical sheep of Group I which was sacrificed soon after *in situ* I^{131} began to diminish as a result of loss of ability of the thyroid either to retain or to concentrate iodine. Figure 2 shows the protein-bound iodine values in an animal of Group II which was fed I^{131} until

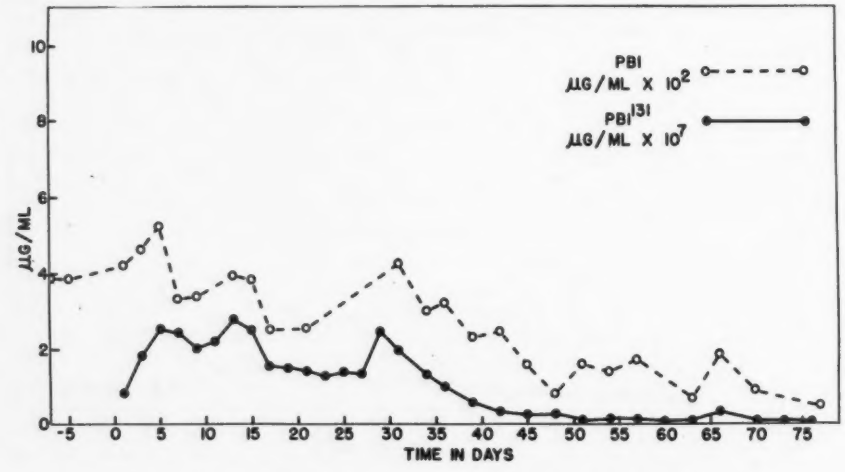

FIG. 3. Serum protein bound iodine in sheep receiving daily oral doses of 480 μc. of I^{131}. Sheep No. 9, Group III.

in situ thyroid I^{131} began to fall. At that time the feedings were discontinued and the viability of remaining thyroid tissue was later investigated by administration of a tracer dose of I^{131}. Figure 3 shows the protein-bound iodine values in the serum of a sheep in Group III which was fed I^{131} throughout the experiment. In all cases the total protein-bound iodine determinations were started several days before the first administration of I^{131}. Zero day on the abscissa represents the first day of I^{131} feeding.

TABLE 1. CONVERSION RATIOS AND IODINE FRACTIONS IN THE SERA OF SHEEP RECEIVING I^{131}

Group No.	Day of "break" in thyroid count after feeding begins	Period in days sera were analyzed	Total PBI $\mu g./ml. \times 10^2$ (average)	PBI^{131} $\mu g./ml. \times 10^5$ (average)	Inorg. I^{131} $\mu g./ml. \times 10^5$ (average)	Conversion ratio (average)
		−6–+30	3.99	4.98*	0.19*	0.96*
	40	32– 40	2.15	8.63	0.15	0.92
		44– 50	1.83	5.97	2.37	0.70
		53– 58	2.23	4.07	5.47	0.45
		−7–+25	3.92	18.0*	0.46*	0.98*
I	35	27– 35	2.95	17.9	1.98	0.89
		38– 43	4.10	16.1	6.10	0.72
		−7–+25	3.80	9.4*	0.33*	0.97*
	35	27– 35	2.95	14.75	0.95	0.94
		38– 44	4.20	12.53	3.60	0.78
		−6–+46	3.37	6.97*	0.76*	0.91*
	58	49– 58	2.00	5.28	2.95	0.67
		61– 64	2.15	4.55	4.35	0.51
		67– 74†	2.17	—	—	—
		−5–+23	4.03	11.72*	0.17*	0.98*
II	34	25– 34	3.90	16.3	1.30	0.92
		37– 46	3.23	11.47	2.55	0.82
		49– 74†	2.53	—	—	—
		−7–+15	3.72	7.63*	0.40*	0.95*
	25	17– 25	2.45	6.05	1.14	0.84
		27– 31	4.26	20.3	6.03	0.71
		34– 74†	1.96	—	—	—
		−6–+26	3.86	15.8*	0.53*	0.98*
	36	28– 36	3.33	13.2	1.42	0.90
		39– 45	3.27	11.5	2.40	0.83
		48– 98	1.90	4.33	5.06	0.44
		−6–+26	3.68	4.50*	0.50*	0.90*
III	36	28– 36	2.30	7.08	1.04	0.87
		39– 45	2.40	6.67	1.53	0.81
		48– 106	2.90	5.00	5.22	0.49
		−7–+15	4.08	25.0*	0.70*	0.97*
	25	17– 25	2.60	14.3	2.94	0.86
		27– 34	3.65	17.7	10.70	0.61
		36– 84	1.58	2.49	10.00	0.32

* Determinations prior to tenth day excluded from average.
† I^{131} feeding stopped at beginning of this period.

Analysis of data from all the experimental sheep (Table 1) seems to establish certain general trends. The total protein-bound iodine levels in all sheep prior to the I^{131} feedings were approximately 4 μg. per 100 ml. serum, but as thyroid damage occurred the values decreased. This is especially evident in sheep which received I^{131} throughout the entire experiment. The serum in sheep of Group II showed a continued fall in total protein-bound iodine after I^{131} feedings were stopped. In most cases, the initiation of I^{131} feedings was immediately followed by temporarily increased total protein-bound iodine values in serum. This suggests that radiation effects on thyroid tissue may bring about a large release of preformed protein-bound iodine into the circulation.

The protein-bound I^{131} levels rose gradually after the feedings were initiated. When damage to the thyroid was sufficiently prolonged and severe, the fall in total protein-bound iodine in the serum was usually accompanied by a decrease in protein-bound I^{131} (Figure 3).

Although Figures 1, 2, and 3 indicate that the total protein-bound iodine values in serum serve as a comparatively sensitive index of thyroid function, the colorimetric procedure used in this type of determination is rather complex and laborious. Various investigators have attempted to evaluate the state of the thyroid by its uptake of I^{131} or by the rate of urinary excretion of this isotope, but they found considerable overlapping of rates in hypothyroidism, euthyroidism, and hyperthyroidism (Werner *et al.*, 1950). In cases of renal insufficiency spurious high levels of blood I^{131}, resulting from a delayed excretion of inorganic I^{131}, are often encountered (Siver *et al.*, 1950). Evidence is available, however, indicating that when I^{131} is administered either by injection or orally, its rate of incorporation into the protein-bound form may serve as an index of thyroid activity, since this conversion rate increases in hyperthyroidism (McConahey *et al.*, 1949), (Freedberg *et al.*, 1949) or if thyrotropin is injected (Chaikoff *et al.*, 1947) and decreases after thyroidectomy. By setting up the ratio:

$$\frac{\text{Protein-bound } I^{131} \text{ in plasma}}{\text{Total } I^{131} \text{ in plasma}}$$

Clark and associates (1949, 1950) were able to study the rate of incorporation of orally administered inorganic I^{131} into protein-bound iodine in patients with various thyroid states. By multiplying this ratio by 100 they obtained a conversion ratio in per cent. In 22 euthyroid patients the conversion ratio ranged from 13 to 42 per cent while in 28 hyperthyroid patients ratios between 45 and 96 per cent were found. Values of 10 per cent or less were considered indicative of hypothyroidism.

Since the conversion ratio appears to be an expression of the synthesiz-

ing ability of the thyroid, this ratio was calculated for all the sheep receiving I^{131}. Values for the representative sheep of each group are shown in Figures 4, 5, and 6. On these same figures are also plotted the uptake of I^{131} by the thyroid as evidenced by external thyroid monitoring.

Analysis of values calculated for sheep in Groups I and III reveals that there was a gradual but progressive fall in conversion ratios indicative of thyroid damage and loss of ability to synthesize and release protein-bound

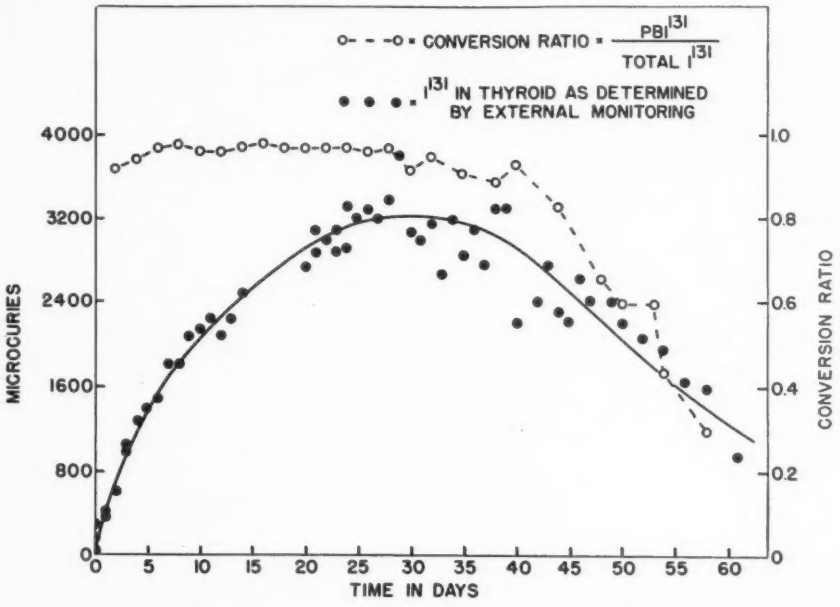

FIG. 4. Comparison of conversion ratios and external thyroid monitoring of sheep receiving daily doses of 480 μc. of I^{131}. Sheep No. 1, Group I.

iodine into the circulation. The lowest conversion ratios were observed in the sheep fed I^{131} over long periods of time (Group III). The conversion ratios calculated for sheep of Group II also decreased, but since in two of these animals the I^{131} feeding period lasted only 40 days, the fall in conversion ratio was less pronounced than in the case of the sheep in Group III.

Figure 5 shows that, after the I^{131} administration was discontinued, the conversion ratios returned to former values. This must not be interpreted to mean, however, that the synthesizing ability of the thyroid was regained. The observed rise in the ratio is merely due to the fact that in the absence of ingested I^{131} the fraction $\frac{\text{protein-bound } I^{131}}{\text{total } I^{131}}$ in serum approaches

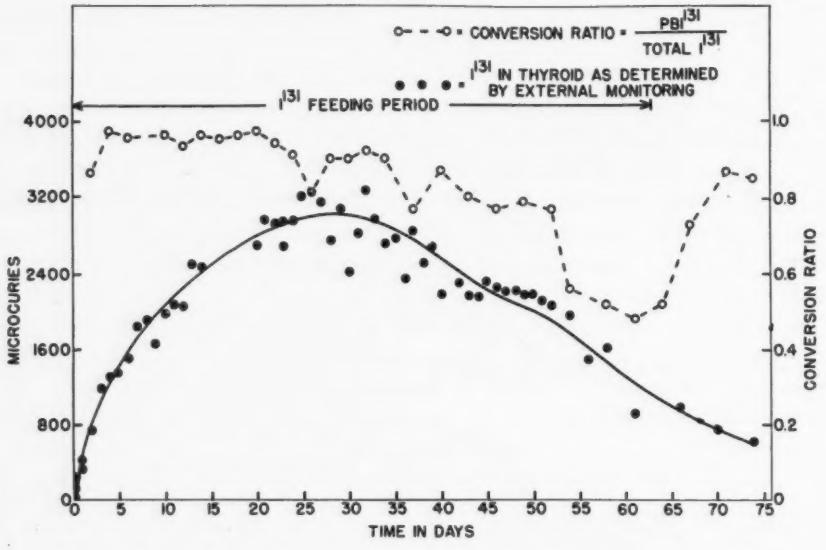

FIG. 5. Comparison of conversion ratios and external thyroid monitoring of sheep receiving daily doses of 480 μc. of I^{131}. Sheep No. 4, Group II.

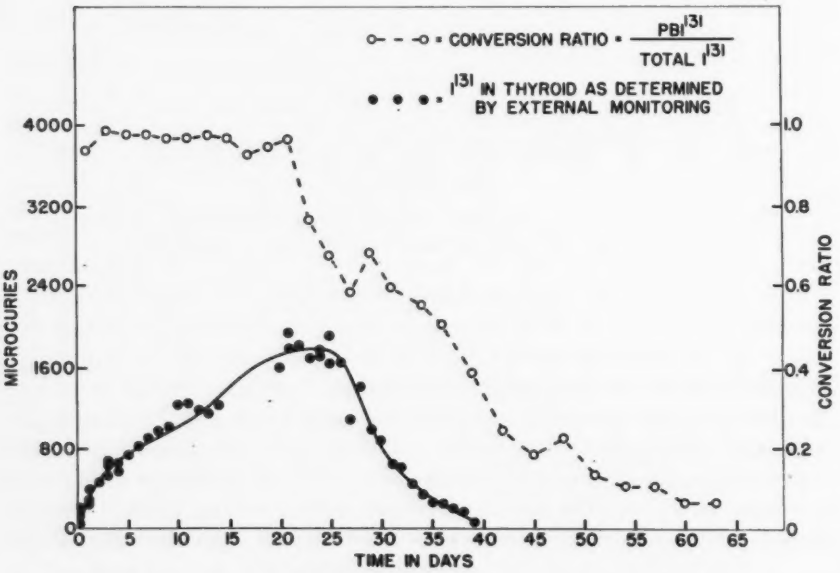

FIG. 6. Comparison of conversion ratios and external thyroid monitoring of sheep receiving daily doses of 480 μc. of I^{131}. Sheep No. 9, Group III.

unity. When the I^{131} feedings were discontinued, the total I^{131} in the serum was observed to decrease to practically negligible levels. That extensive damage to the thyroid had occurred in all sheep of Group II was proven by the administration of 60–72 μc. doses of I^{131} seven weeks after the last previous I^{131} feeding. Negligible synthesis of protein-bound I^{131} was detectable at this time.

A condensation of data on conversion ratios and on the various iodine fractions in the serum of all experimental sheep at representative periods of time preceding and following "the break" or decrease of *in situ* thyroidal I^{131}, as evidenced by external thyroid monitoring, can be found in Table 1. Although the majority of the animals receiving 480 μc. of I^{131} per day showed a diminution of *in situ* thyroidal radioiodine within 34 to 40 days after feedings were started, two sheep were considerably more sensitive to radiation damage, while one did not begin to lose the ability to deposit I^{131} in the thyroid until 58 days after zero day. Before "the break" the protein-bound I^{131} levels in sheep of Groups I and III were much higher than the inorganic I^{131} levels. Several weeks after "the break" this ratio was reversed. When the data on conversion ratios and the quantity of *in situ* thyroidal I^{131} were compared it was found that in 8 sheep the "break" occurred from 3–8 days after the decrease in conversion ratio, while in the ninth sheep both changes occurred on the same day. This suggests that the synthesizing function of the thyroid is more sensitive to radiation damage than the ability of the thyroid to concentrate iodine as determined by external counting.

SUMMARY

Nine Suffolk ram lambs received daily oral doses of 480 μc. of I^{131} over periods of time ranging from 31 to 90 days. Radiation damage to the synthetic function of the thyroid was investigated by measuring the total protein-bound iodine, the protein-bound I^{131}, and the inorganic I^{131} in the serum of these animals. The iodine uptake was investigated by external monitoring.

1. The total protein-bound iodine levels and the conversion ratios, $\frac{\text{protein-bound } I^{131}}{\text{total } I^{131}}$ in serum, which express the ability of thyroid tissue to convert inorganic I^{131} to the organic form, decreased as a result of thyroid damage due to radiation.

2. Interference with the synthetic function of the thyroid, as evidenced by a fall in conversion ratio, preceded by several days the inhibition of iodine uptake of the gland as measured by external thyroid monitoring. The determination of conversion ratios may prove to be a convenient procedure for detecting early radiation damage to the thyroid.

Acknowledgements

Sincere appreciation is extended to Miss Patricia L. Hackett, Mr. K. E. Herde, and Dr. L. K. Bustad for their assistance and advice during the course of this work.

REFERENCES

BARKER, S. B. AND M. J. HUMPHREY: *J. Clin. Endocrinol.* **10:** 1136. 1950.
CHAIKOFF, I. L., A. TAUROG AND W. O. REINHARDT: *Endocrinology* **40:** 47. 1947.
CLARK, D. E., R. H. MOE AND E. E. ADAMS: *Surgery* **26:** 331. 1949.
FELLER, D. D., I. L. CHAIKOFF, A. TAUROG AND H. B. JONES: *Endocrinology* **45:** 464. 1949.
FREEDBERG, A. S., A. URELES AND S. HERTZ: *Proc. Soc. Exptl. Biol. Med.* **70:** 679. 1949.
HEALY, J. W., L. C. SCHWENDIMAN AND R. C. THORBURN: *Document* **HW-18258:** 1950.
MCCONAHEY, W. M., F. R. KEATING AND M. H. POWER: *J. Clin. Invest.* **28:** 191. 1949.
PALMER, R. F.: *Document* **HW-21177:** 1951.
SHELINE, G. E. AND D. E. CLARK: *J. Lab. Clin. Med.* **36:** 450. 1950.
SILVER, S. AND M. H. FIEBER: *Proc. Soc. Exptl. Biol. Med.* **75:** 570. 1950.
WERNER, S. C., H. B. HAMILTON, E. LEIFER AND L. D. GOODWIN: *J. Clin. Endocrinol.* **10:** 1054. 1950.

SOME BIOLOGICAL PROPERTIES OF DIFFERENT ESTERS OF DESOXYCORTICOSTERONE

ROBERT GAUNT, JAMES H. LEATHEM, CONSTANCE HOWELL AND NANCY ANTONCHAK

From the Research Laboratories, Ciba Pharmaceutical Products, Inc., Summit, New Jersey

FOR many years the acetate of desoxycorticosterone (DCA) has occupied a preeminent position as the 11-desoxycorticoid of widest use. The compound is generally administered in oil solution and the effect of a single injection is of moderate duration. A quick-acting, water-soluble desoxycorticosterone—desoxycorticosterone glucoside—has been prepared; it is very effective in reviving dogs from crises of adrenal insufficiency (Meier, Gysel and Müller, 1944; Swingle, Perlmutt, Seay and Collins, 1952). We report here obervations on esters of desoxycorticosterone which, on the other hand, are markedly longer acting than DCA.

It has been known for some time that esters of desoxycorticosterone other than its acetate had more prolonged activity and this was notably true of the benzoate and palmitate (Miescher, Fischer and Tschopp, 1938). More recently the trimethylacetate of desoxycorticosterone has been prepared by Wieland, Heer, Schmidlin and Miescher (1951). The preliminary results of these authors indicated a long duration of activity when this compound was given in oil solution to rats and dogs. This prompted a further study of the trimethylacetate and other desoxycorticosterone esters in varying dosages and means of administration both in animal and clinical laboratories. The pharmacological properties of the trimethylacetate have been explored in animals by Gross and Tschopp (1952). The phenylacetate and β-cyclopentylpropionate of desoxycorticosterone were prepared in this laboratory.[1] These and the trimethylacetate have been studied in both dogs (Swingle, Collins, Barlow and, Fedor, 1952) and rats (Gaunt, Leathem, Howell and Antonchak, 1952). Clinical studies on the trimethylacetate and phenylacetate have shown that when given as single injections of microcrystalline aqueous suspension, Addisonian patients can be maintained for long periods of time (Frawley and Forsham, 1951). Thorn and Jenkins (1952) found in a study of 35 patients that injections

Received for publication February 1, 1952.

[1] Dr. A. St. Andre made the desoxycorticosterone phenylacetate, β-cyclopentylpropionate, and most of the desoxycorticosterone trimethylacetate used here. Dr. P. Ulshafer prepared the Compound S.

of 60 to 90 mg. of the trimethylacetate sufficed for periods of 4 to 6 weeks. Soffer and Sorkin (personal communication) obtained almost identical results in 10 cases: 45 to 100 mg. maintained patients from 4 to 8 weeks (average, 5.5 weeks). They could not distinguish between the effects of the trimethylacetate and phenylacetate. Doctor C. W. Lloyd and associates have used the trimethylacetate in 8 cases. Doses of 45 to 125 mg. prevented the appearance of Addisonian symptoms for two weeks to two months.

In this report we have compared in certain respects the effects of various esters of desoxycorticosterone with those of free desoxycorticosterone, cortisone acetate and 17-hydroxy-11-desoxycorticosterone acetate (Reichstein's Compound S). In view of the fact that our primary interest was to determine duration of activity, all of the results reported here with the exception of progestational tests are based on the effects of a single subcutaneous injection of the steroid in question.

OBSERVATIONS

Life-Maintenance and Growth (Table 1). Immature male rats, approximating 60 gm. body weight, were adrenalectomized and given immediately a single subcutaneous dose of the steroid to be tested. In one series the compounds were given in oil solution. Cortisone and Compound S were not included in this group because of their relative insolubility in oil. In another series, the steroids were used as microcrystalline aqueous suspensions. The cortisone acetate employed was Merck's Cortone acetate, with a crystal size of 5 to 10 micra. The desoxycorticosterone trimethylacetate had an average crystal size of 40 to 50 micra and all other preparations an average crystal size of 30 to 40 micra. The effects on survival are shown in Table 1. Included in this table also are figures on the average weight gain per day based on initial and terminal weights and on the maximal weight gain whenever that might have occurred. Typically the animals reached a certain maximal weight and then declined to a lower weight before death.

a) *Oil Solutions.* All of the corticoids studied extended the life span of adrenalectomized rats with the possible exception of the lower doses of free desoxycorticosterone. It is noteworthy that the "weak" compounds, even in doses which had little effect on survival, supported appreciable weight increments before death. In fact, during the short period of its effectiveness, 5 mg. of free desoxycorticosterone caused the most rapid daily weight gains of any compound studied in oil solution. Presumably this is a manifestation of rapid absorption.

The trimethylacetate was clearly the most effective ester in oil solution at all dose levels tested. This might be due solely to slow absorption. A study of the growth response to the compound, however, makes such a conclusion uncertain if not, indeed, unlikely. If its superiority in life-

TABLE 1. MEAN FIGURES SHOWING SURVIVAL AND GROWTH OF ADRENALECTOMIZED RATS GIVEN SINGLE SUBCUTANEOUS INJECTIONS OF VARIOUS CORTICOIDS

Oil solution					Aqueous suspension					
No. rats	Dose, mg.	Wt. gain, gm./day	Maximal wt. gain, gm.	Survival, days	No. rats	Dose, mg.	Wt. change, gm./day[1]	Maximal wt. gain, gm.[1]	Survival, days	% surviving >80 days
Desoxycorticosterone										
7	1	1.35	14.6	8.9	10	1	1.98	27.0	12.8	10
11	2	1.59	21.7	9.6						
13	5	3.10	37.6	10.9	10	5	1.70	33.2	13.0	20
Desoxycorticosterone acetate										
10	1	1.42	17.7	9.8	10	1	1.55	32.0	13.0	30
12	2	1.50	19.0	10.8						
10	5	1.47	24.5	11.4	10	5	1.76	45.3	18.0	10
Desoxycorticosterone trimethylacetate										
7	1	1.66	48.0	18.4	10	1	2.87	83.5	25.0	60
13	2	2.27	65.8	25.9						
12	5	2.18	77.2	28.6	10	5	3.02	198.0	55.0	50
Desoxycorticosterone phenylacetate										
8	1	1.51	18.1	10.0	10	1	2.32	56.0	19.3	40
6	2	1.89	24.2	10.7						
13	5	1.99	25.6	10.5	10	5	2.28	122.0	47.0	30
Desoxycorticosterone-β-cyclopentylpropionate										
9	1	2.13	36.8	12.7	10	1	2.13	30.7	11.6	0
15	2	1.92	42.0	17.0						
16	5	1.98	49.2	20.4	10	5	1.74	40.0	17.0	30
Cortisone acetate										
—	—	—	—	—	10	1	0.50	9.3	8.8	10
—	—	—	—	—	10	5	0.44	13.1	15.0	20
17-hydroxy-11-desoxycorticosterone acetate (Compound S)										
—	—	—	—	—	10	1	1.35	21.8	12.0	0
—	—	—	—	—	17	2	1.80	28.6	13.1	18
—	—	—	—	—	15	5	1.95	60.0	26.7	13
Controls—Untreated										
34	0	0.28	4.0	7.1	10	0	−0.03	4.4	6.4	0

[1] Animals that died only.

maintenance were due entirely to slow absorption, one might expect that its effect on growth would be less than that of the presumably more rapidly absorbed shorter-acting acetate. It is noted, however, that trimethylacetate is superior to the acetate both in the maintenance of growth and life.

b) *Aqueous Suspensions.* In nearly all cases the corticoids maintained

life for longer periods when given in aqueous suspension than in oil solution. The most effective compound was again desoxycorticosterone trimethylacetate. In marked distinction to their relative action in oil solution, however, the phenylacetate approached the trimethylacetate in effectiveness. (In dogs the two were apparently equal in effectiveness. Swingle *et al.*, 1952). The β-cyclopentylpropionate was exceptional in being no more active than in oil solution; in aqueous suspension, it was if anything less active than Compound S. Cortisone acetate, as expected, exerted a definite but weak effect on survival and failed to support appreciable growth. The growth-promoting properties of other compounds were closely related to their ability to maintain life.

c) *Relation of Adrenal Accessories to Survival* (Table 1). It has been known for a long time (Gaunt and Gaunt, 1934) that, at least in certain colonies of rats, any therapy which prolongs survival for long periods after adrenalectomy results in the indefinite survival of some animals apparently due to the fact that time is allowed for the development of adrenal accessories. Whether these accessories represent hypertrophied adrenal rests *per se*, or the differentiation of cortical tissues from coelomic mesothelium is an unsettled point (McFarland, 1945; Gaunt and Eversole, 1949).

In our studies with oil solutions occasional animals which survived indefinitely were discarded without attempts at histological identification of suspected cortical tissue. In the series receiving steroids in aqueous suspension, however, the number of animals surviving indefinitely became too large to ignore. In fact, their number became a rough measure of the effectiveness of the compounds being tested. Thus, the greatest number of "indefinite" survivals occurred in the groups receiving either trimethylacetate or phenylacetate esters.

In view of its obvious importance in this experiment, histological identification was made of suspected adrenal tissue in all cases. All surviving animals were sacrificed at approximately 80 days after adrenalectomy. In some cases the cortical accessories found were very small—0.5–1.0 mm. in diameter. In a few cases (possible incomplete removal?) the mass of tissue approximated the size of a normal adrenal. Others were intermediate between these extremes.

While the possibility cannot be excluded in some cases that the adrenal tissue seen in animals surviving indefinitely was due to inadvertent failure to remove all of the main adrenal glands, that certainly was not the case in most instances. This conclusion is based on the following considerations: (1) Notation was always made of the fact if there was any reason to suspect that complete adrenal removal was not achieved at operation. (2) The accessory tissue found was on the left side in 80 per cent of the

cases, as previously found (Gaunt and Gaunt, 1934), although that is the side on which total excision of visible adrenal tissue is most easily accomplished. (3) Essentially the same results were obtained by three different experienced operators. (4) In 5 per cent of the cases surviving, no adrenal tissue was found at autopsy, although had a regenerated fragment of the main gland been present, it should certainly have been located. (5) Some of these accessories were of barely macroscopic size, whereas in the time available a fragment of the main gland would have been expected to regenerate to considerable size with all effective therapies. (6) Finally, and again as previously found (Gaunt and Gaunt, 1934), the observed accessories were oftentimes at sites—generally near the junction of the left adrenal and renal vein—where their removal at a routine adrenalectomy would have been impossible.

(*Addendum at proof-reading:* In a repetition of the survival experiment described above, 1 mg. doses in aqueous suspension of the indicated compounds were given at the time of adrenalectomy in groups of 10 rats. Mean survival in days and per cent of survivals over 80 days respectively were as follows: DC-acetate, 11.8, 10%; DC-phenylacetate, 21.8, 30%; DC-trimethylacetate, 35.5, 20%; untreated, 6.1, 0%.)

Inhibition of Release of ACTH (Table 2). The various steroids were

TABLE 2. MEAN FIGURES SHOWING EFFECT OF VARIOUS CORTICOIDS ON ADRENAL WEIGHT[1]—ACTH-SUPPRESSING ACTIVITY

No. rats	Initial body wt.	Left adrenal weight before treatment	Right adrenal weight 5 days after treatment	Right adrenal weight 10 days after treatment
Desoxycorticosterone acetate				
10	70.5	11.14	10.71	
10	71.6	11.99		13.07
Desoxycorticosterone trimethylacetate				
10	66.9	12.33	11.89	
10	72.7	12.24		13.47
17-hydroxy-11-desoxycorticosterone acetate (Compound S)				
10	70.6	13.17	12.33	
10	72.4	12.90		13.08
Cortisone acetate				
10	69.4	12.98	9.44	
10	72.6	12.33		12.74
Controls—untreated				
10	70.2	12.61	13.45	
10	72.1	12.16		14.04

[1] Adrenal weights are expressed as mg. per 100 gm. body weight.

tested for their ability, again after a single subcutaneous injection of 5 mg. in aqueous suspension, to inhibit the release of ACTH from the anterior pituitary in immature female rats, approximating 65 gm. body weight. The criterion used was that of preventing the expected increase in the size of the right adrenal after left adrenalectomy. The compounds tested were injected at the time of left adrenalectomy and the effects observed at five and ten days after treatment. The weight of the right adrenal of treated animals could then be compared with that of their own left adrenals and with the right adrenals of control animals subjected to left adrenalectomy only.

It is noted that at both 5 and 10 days mean right adrenal weights were at least slightly less than those of untreated controls. As expected, cortisone acetate was the most effective corticoid in suppressing ACTH activity. If the figures for right adrenal weights are arranged in order of magnitude adjacent means are not significantly different at a 5 per cent level of probability; they are different at less than a 10 per cent level of probability. Alternate means differ significantly by conventionally acceptable criteria (i.e. $P = <.05$). These results suggest that all of the compounds, under the conditions of administration used here, exerted a mild pituitary-depressing action. Since DC-trimethylacetate is being used clinically in the form of single large and infrequent doses, it is of some interest to know that no marked and immediate pituitary suppression is to be expected.

Effects on Water and Chloride Excretion (Table 3). It is well-known that in the absence of cortical hormones there is a deficient diuretic response to a water load, a susceptibility to water intoxication that is not entirely due to the deficient water diuresis, and that under most circumstances desoxycorticosterone causes a retention of sodium and chloride (Gaunt *et al.*, 1949). The effects of the various corticoids on these phenomena were observed in experiments conducted as follows. Male rats, approximating 150 gm. body weight, were adrenalectomized and given immediately an injection of 5 mg. of the steroid to be tested in aqueous suspension. The animals were then fasted for 18 hours (drinking water allowed) after which three doses of water, 3 ml. per 100 sq. cm. of body surface, warmed to body temperature, were given by stomach tube at hourly intervals. Measurements were made of the rate of water diuresis, changes in body temperature (a measure of water intoxication—Gaunt, 1944) and of the total chloride excreted during the experiment. Rectal temperatures were taken with an iron-constantan junction thermocouple. The figures are based upon four experiments in which two animals per group were run simultaneously. The same experiment was repeated on the same animals one week later. Chloride excretion was not measured in the latter experiment because the presence of water intoxication resulted in frequent contamination of urine samples by diarrhea.

In tests conducted at 18 hours after adrenalectomy and 18 hours after injection of the microcrystalline suspensions, only cortisone acetate relieved the deficient diuretic response to water characterizing the untreated adrenalectomized rats. The weakness of DCA in maintaining water diuresis both in adrenal deficient animals and men is well-known (Gaunt *et al.*,

TABLE 3. MEAN FIGURES SHOWING THE EFFECT OF VARIOUS CORTICOIDS ON RESPONSE OF ADRENALECTOMIZED RATS TO WATER LOADING

18 hours after adrenalectomy					7 days after adrenalectomy		
No. rats	% water load excreted at 5th hr.	Body temp. loss F°	Chloride excreted as NaCl		No. rats	% water load excreted at 5th hr.	Body temp. loss F°
			mg./ml.	Total mg.			
Untreated adrenalectomized controls							
8	53	−1.0	0.35	5.0	6	6	−4.8
Untreated intact controls							
10	81	—	0.38	7.9	—	—	—
Cortisone acetate							
8	83	−0.6	0.39	7.4	8	12	−5.3
Desoxycorticosterone acetate							
8	50	−0.4	0.18	2.3	8	20	−2.2
Desoxycorticosterone trimethylacetate							
7	52	−1.0	0.15	2.2	7	17	−2.6
Desoxycorticosterone phenylacetate							
8	52	−1.1	0.36	5.0	8	17	−3.6
Desoxycorticosterone-β-Cyclopentylpropionate							
8	54	−0.7	0.15	2.4	8	13	−4.5
17-Hydroxy-11-Desoxycorticosterone acetate (Comp. S)							
8	60	−0.8	0.12	2.4	8	12	−5.3

1949; Gross and Meier, 1951) but DCA in oil administered in other ways is mildly active (Eversole *et al.*, 1942; Gross and Meier, 1951).

Compound S and all of the desoxycorticosterone esters, excepting possibly the phenylacetate, reduced chloride excretion. The high mean chloride excretion of the phenylacetate-treated rats was due to an exceptionally high chloride output of two animals, likely an aberrant performance rather than a manifestation of a different action of this ester. Excepting cortisone, which exerted its expected effect in causing a high chlo-

ride and water excretion, no consistent difference between the other compounds was apparent.

When similar tests were repeated on the same animals one week after adrenalectomy and one week after treatment diuresis was sluggish and some degree of water intoxication (temperature fall) occurred in all groups. Although the data suggest that the four desoxycorticosterone esters may have exerted a slight replacement value such a conclusion could not be established statistically. The effect of cortisone clearly had largely worn off, probably because of complete absorption.

Progestational Activity (Table 4). Previous investigations have demon-

TABLE 4. PROGESTATIONAL ACTIVITY OF DESOXYCORTICOSTERONE ESTERS IN ESTROGEN-PRIMED IMMATURE RABBITS

Compound	No. rabbits	Total dose, mg.	Endometrial response
Desoxycorticosterone	6	5	1
DC-acetate	4	5	1 or 2
DC-phenylacetate	6	5	2
DC-β-cyclopentylpropionate	4	5	2 or 3
DC-trimethylacetate	4	5	1 or 2
DC-trimethylacetate	3	10	1 or 2

strated that desoxycorticosterone and its acetate will induce a progestational endometrium after pre-treatment with estrogen in immature rabbits (Miescher, Fischer and Tschopp, 1938; Holweg, 1939; van Heuverswyn *et al.*, 1939; Wells and Greene, 1939; de Fremery and Spanhoff, 1939; Leathem and Crafts, 1940) and in spayed adrenalectomized cats (Leathem and Crafts, 1940). The manner in which other desoxycorticosterone esters would influence the endometrium and an approximation of dosage relationships was deemed of interest in this study. Female rabbits (1 kg.) were given daily subcutaneous injections of 0.015 mg. of estradiol benzoate for 8 days followed by 5 daily injections of the steroid to be studied. Twenty-four hours after the last injection, the rabbits were sacrificed and the uterus prepared for histological study of the endometrium.

The data presented in Table 4 shows that all the desoxycorticosterone esters studied here induced a definite progestational endometrium and that at comparable dosages the phenylacetate and cyclopentylpropionate were consistently more active than the acetate. It is known, however, that a 2 plus endometrium is obtained with a total dosage of 0.5 mg. of progesterone. Obviously then the compounds studied here were relatively weak progestogens.

DISCUSSION

It is well-known that the biological activity of steroid molecules can be greatly modified by different types of esterification and by the physical form in which they are administered. Most studies relating to these points

have been conducted on the sex hormones. It is apparent from the work reported here and elsewhere that in oil solution the trimethylacetate of desoxycorticosterone has a duration of effective activity much greater than that of the commonly used acetate. It remains to be established with certainty whether that difference is due to a slower absorption of the former but as pointed out above there is reason to question whether the explanation is that simple. It is of interest that compounds such as free desoxycorticosterone, which after a single injection have only a slight effect on survival, nevertheless do exert a marked influence on growth.

When the compounds used here were administered as microcrystalline aqueous suspensions their duration of activity was generally but not always (e.g. desoxycorticosterone β-cyclopentylpropionate) greatly increased. It is clear from this and other work that compounds such as desoxycorticosterone trimethylacetate can be given in rats, dogs and men in a form (microcrystalline aqueous suspension) in which a single injection will produce prolonged activity without evidence of acute overdosage immediately after administration. Very quick and transient effects, on the other hand, can be obtained by using the water-soluble desoxycorticosterone glucoside, and effects of medium duration by giving desoxycorticosterone acetate in oil. Of the preparations now available the longest activity results from the use of DCA pellets, but their administration is less expedient than an injection. This array of preparations permits considerable purposeful latitude in the design of experimental or clinical regimens.

It is of interest that a compound such as desoxycorticosterone phenylacetate, obviously weak in oil solution, is highly effective in crystalline suspension, whereas the β-cyclopentylpropionate is of equal activity in the two forms. The data presented here offer no interpretation of such phenomena. The relatively weak growth response to the phenylacetate in oil solution is not entirely compatible with the idea that its greater effectiveness in microcrystalline form is entirely a matter of slower absorption.

In all of our observations Compound S behaved like a weak desoxycorticosterone. Others have obtained similar results (reviewed by Gross and Meier, 1951). In the circumstances studied it was devoid of activity only in its ability to stimulate water diuresis and again in this respect it mimicked the desoxycorticosterones. Probably like DCA it could be given in some other dose and manner that would affect water excretion at least weakly. Such a prediction is substantiated by the results of Corcoran (1950) who observed some increase in the daily water exchange of rats given 5 mg. of Compound S daily.

SUMMARY

Some effects were observed in adrenalectomized rats of single injections of the following compounds in oil solution: desoxycorticosterone, DC-

acetate, DC-trimethylacetate, DC-phenylacetate and DC-β-cyclopentylpropionate. The same compounds and in addition cortisone acetate and 17-hydroxy-11-desoxycorticosterone acetate (Compound S) were studied in microcrystalline aqueous suspensions after single injections.

In oil solution, the duration of activity of DC-trimethylacetate was the greatest of the compounds studied. In aqueous suspension, the effectiveness of most of the compounds was enhanced and the test animals frequently developed adrenal accessories. Desoxycorticosterone trimethylacetate in aqueous suspension was again the most active compound in supporting life and growth, although its effectiveness in these respects was approached by desoxycorticosterone phenylacetate.

Single injections of all the compounds in crystalline suspension, except cortisone, failed to stimulate water diuresis, suppressed ACTH activity slightly if at all, but generally caused chloride retention. Cortisone stimulated water diuresis, suppressed ACTH activity markedly but did not cause chloride retention. The effects of Compound S acetate were like those of a weak DCA.

REFERENCES

CORCORAN, A. C.: *Pituitary-Adrenal Function, Amer. Assoc. Adv. Sci.*, Washington, P. 183. 1950.

DE FREMERY, P. AND R. W. SPANHOFF: *Acta brev. neerl.* **9**: 79. 1939.

EVERSOLE, W. J., R. GAUNT AND E. C. KENDALL: *Am. J. Physiol.* **135**: 378. 1942.

FRAWLEY, T. F. AND P. H. FORSHAM: *J. Clin. Endocrin.* **11**: 772. 1951.

GAUNT, R.: *Endocrinology* **34**: 400. 1944.

GAUNT, R., J. H. BIRNIE AND W. J. EVERSOLE: *Physiol. Rev.* **29**: 281. 1949.

GAUNT, R. AND W. J. EVERSOLE: *Ann. N. Y. Acad. Science* **50**: 511. 1949.

GAUNT, R. AND J. H. GAUNT: *Proc. Soc. Exp. Biol. & Med.* **31**: 490. 1934.

GAUNT, R., J. LEATHEM, C. HOWELL AND N. ANTONCHAK: *Fed. Proc.* **11**: 53. 1952.

GROSS, F. AND R. MEIER: *Schweiz. med. Wchnschr.* **41**: 1013. 1951.

GROSS, F. AND T. TSCHOPP. *Experientia.* **8**: 75. 1952.

HOHLWEG, W.: *Ztschr. F. Gynak.* **63**: 1143. 1939.

LEATHEM, J. H. AND R. C. CRAFTS: *Endocrinology* **27**: 283. 1940.

MCFARLAND, W. E.: *Anat. Rec.* **93**: 233. 1945.

MEIER, R., H. GYSEL AND R. MULLER: *Schweiz. med. Wchnschr.* **74**: 93. 1944.

MIESCHER, K., W. H. FISCHER AND T. TSCHOPP: *Nature* **142**: 435. 1938.

SWINGLE, W. W., E. COLLINS, G. BARLOW AND E. FEDOR: *Am. J. Physiol.* In press. 1952.

SWINGLE, W. W., J. PERLMUTT, P. SEAY AND E. COLLINS: *Am. J. Physiol.* **168**: 825. 1952.

THORN, G. W. AND D. JENKINS: *Schweiz. med. Wchnschr.* In press. 1952.

VAN HEUVERSWYN, J., V. J. COLLINS, W. L. WILLIAMS AND W. J. GARDNER: *Proc. Soc. Exp. Biol. & Med.* **41**: 552. 1939.

WELLS, J. A., AND R. R. GREENE: *Am. J. Physiol.* **126**: 652. 1939.

WIELAND, P., J. HEER, J. SCHMIDLIN AND K. MIESCHER: *Helv. Chim. Acta* **34**: 354. 1951.

ADRENAL ASCORBIC ACID CHANGES IN THE GONADECTOMIZED GOLDEN HAMSTER FOLLOWING A SINGLE INJECTION OF DIETHYLSTILBESTROL

BERNARD C. WEXLER

Department of Anatomy, Stanford University, School of Medicine, Stanford, California

CHRONIC treatment of golden hamsters with diethylstilbestrol (Kirkman and Bacon, 1950), with estradiol (Kirkman, 1951) is followed by the appearance of malignant renal tumors in males, while females tend to show kidney damage similar to that found in glomerulonephritis (Matthews, Kirkman and Bacon, 1947; Kirkman and Bacon, 1950). It is possible that one or both of these pathological conditions may follow a stilbestrol-induced disturbance of the normal pituitary-adrenal relationship.

Using adrenal ascorbic acid alterations as evidence for ACTH-release (Sayers, 1950), it has been found that following a single injection of stilbestrol there is a marked decrease of vitamin C in the female hamster but only a moderate decrease in the male (Wexler, 1951). In order to study this marked sex difference in adrenal response, the above experiment (Wexler, 1951) was repeated using gonadectomized animals.

METHODS

Hamsters (Cricetus auratus) were gonadectomized at 50 days of age, and at 90 days were divided into two separate groups according to sex. Each animal was given an intraperitoneal injection of 0.06 mg. of diethylstilbestrol (Lilly) in $\frac{1}{4}$ cc. of cottonseed oil and sacrificed by decapitation 0, $\frac{1}{3}$, 1, 3, 6, 12, 24, 36, 48 and 96 hours later. At the time of sacrifice the adrenal glands were removed as quickly as possible, trimmed free of fat, weighed to the nearest 0.01 mg. on a Roller-Smith torsion balance, homogenized, and analyzed for their ascorbic acid content according to the method of Bessey (1938). Each homogenate contained a pool of one adrenal from each of two animals.

RESULTS

Quantitative chemical analysis for adrenal ascorbic acid in the golden hamster showed marked increase following gonadectomy (cf. Tables 1, 2 and Figures 1, 2). The adrenal ascorbic acid content in castrated and spayed hamsters was twice that found in intact males and females.

The adrenal ascorbic acid content of the spayed hamster underwent

Received for publication July 5, 1951.

Present address: Baxter Laboratories, Morton Grove, Illinois.

TABLE 1. CHANGES IN ADRENAL ASCORBIC ACID FROM SPAYED AND NORMAL FEMALE HAMSTERS AT VARIOUS INTERVALS FOLLOWING A SINGLE INJECTION OF 0.06 MG. OF DIETHYLSTILBESTROL

Time (hours)	No. of animals	Average adrenal weight (mgs.)	Average body weight (Gms.)	Average mgs. ascorbic acid per 100 gms. adrenal wt. with S.E.M.	*p* value
Spayed					
0	16	6.4	129	253 ± 23.9	Normal
½	8	6.9	126	160 ± 16.5	.0014
1	8	5.4	121	184 ± 10.2	.0080
3	8	5.1	117	209 ± 16.9	.1136
6	8	5.4	112	251 ± 9.9	Normal
12	8	5.9	112	243 ± 26.1	Normal
24	8	4.9	118	237 ± 7.3	Normal
36	8	5.0	111	243 ± 32.9	Normal
48	8	3.7	109	256 ± 28.9	Normal
96	8	4.5	115	252 ± 17.0	Normal
Normal					
0	16	8.9	125	115 ± 2.1	Normal
½	8	8.4	110	92 ± 3.8	.0001
1	8	9.3	125	71 ± 1.2	.0001
3	8	11.0	130	95 ± 3.7	.0001
6	8	10.3	110	165 ± 17.4	.0004
12	8	8.5	117	132 ± 10.4	.1096
24	16	9.5	114	124 ± 16.3	.5823
36	8	8.7	124	114 ± 3.1	Normal
48	8	8.5	144	114 ± 4.8	Normal

TABLE 2. CHANGES IN ADRENAL ASCORBIC ACID FROM CASTRATE AND NORMAL MALE HAMSTERS AT VARIOUS TIME INTERVALS FOLLOWING A SINGLE INJECTION OF 0.06 MG. OF DIETHYLSTILBESTROL

Time (hours)	No. of animals	Average adrenal weight (mgs.)	Average body weight (Gms.)	Average mgs. ascorbic acid per 100 gms. adrenal wt. with S.E.M.	*p* value
Castrate					
0	16	8.1	135	204 ± 23.4	Normal
½	8	8.9	122	152 ± 13.6	.0574
1	8	7.7	115	144 ± 12.1	.0232
3	8	7.5	112	152 ± 14.2	.0588
6	8	6.9	99	219 ± 3.9	.5287
12	8	7.3	107	199 ± 11.6	Normal
24	8	7.3	118	199 ± 23.4	Normal
36	8	6.9	105	191 ± 19.4	Normal
48	8	6.7	103	207 ± 10.3	Normal
96	8	6.5	111	201 ± 11.1	Normal
Normal					
0	16	12.9	116	109 ± 2.1	Normal
½	12	12.8	118	95 ± 2.4	.0001
1	20	12.5	120	92 ± 1.1	.0001
3	12	13.1	115	117 ± 3.9	.0719
6	8	12.2	112	111 ± 3.7	Normal
12	8	13.6	107	97 ± 1.3	.0012
48	8	12.3	117	98 ± 2.0	.0002
96	4	10.4	109	112 ± 9.0	Normal

an abrupt fall within 30 minutes following the injection of stilbestrol. This was also the point of maximum depletion of ascorbic acid, a depletion of 63.2% of the zero hour percentage (Figure 2). The ascorbic acid content then increased so that by 6 hours it had returned to the level normal for the spayed animal and remained at this level for the duration of the experiment. There was no compensatory increase above normal as has been found in the intact hamster (Figures 1 and 2), the guinea pig and the rat (Long, 1947). The curve for the ascorbic acid values for spayed hamsters

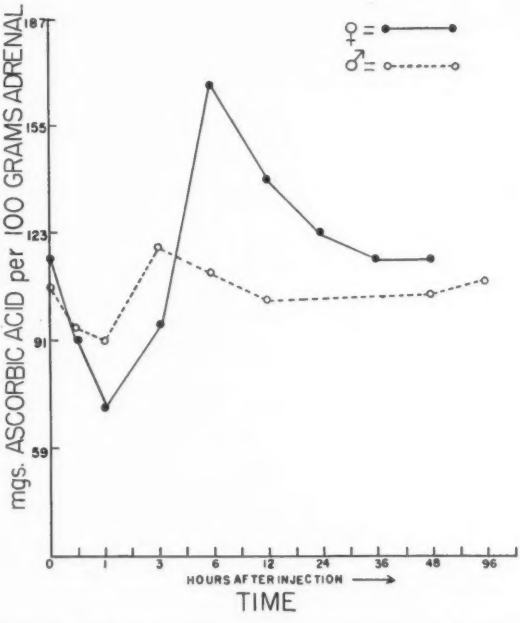

FIG. 1. Changes in adrenal ascorbic acid in the intact golden hamster following a single injection of 0.06 mg. of diethylstilbestrol.

was not only very similar in contour to that for the castrates but also remained consistently at a higher level. This difference in response between spayed and castrated hamsters was found by statistical analysis to be highly significant, the p value being 0.0001.

In the intact male hamster a single injection of stilbestrol failed to elicit the typical changes, mentioned above, in adrenal ascorbic acid shown to be indicative of ACTH-release in the rat and guinea pig. Castration, however, appears to remove this blockage of ACTH-release, for within 1 hour after injection of stilbestrol the ascorbic acid fell more precipitously

in the castrated than in the spayed hamster. This drop in ascorbic acid in the castrate was 70.5% of the zero hour percentage (Figure 2). The depletion-repletion curve for the castrate was very similar to that of the spayed hamster, except that in the former the reaction occurred at a lower level of ascorbic acid content.

In the normal male the adrenal gland weighs more than in the normal

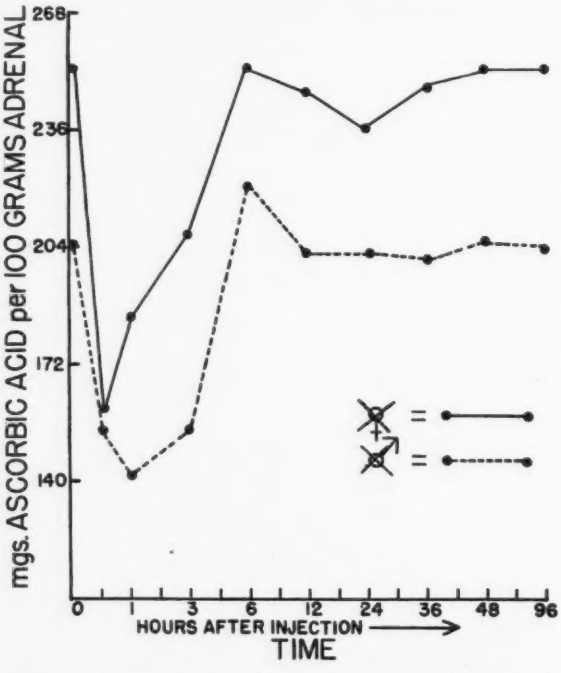

FIG. 2. Changes in adrenal ascorbic acid in the gonadectomized golden hamster following a single injection of 0.06 mg. of diethylstilbestrol.

female hamster (Peczenik, 1943). Peczenik also claims that gonadectomy causes a reduction of adrenal weight in the male and an increase in the female hamster. In this experiment, gonadectomy caused a lowering of both male and female adrenal weight (cf. Discussion).

DISCUSSION

It is interesting to note that gonadectomy in the hamster causes a readjustment in adrenal ascorbic acid levels in both male and female to twice the value found in intact hamsters. Perhaps further investigation of this

will yield more information concerning gonad-adrenal relationships. Papers by Bourne and Zuckerman (1940), Schilling and Lacquer (1941), Selye and Albert (1942), Selye, Rowley, and Hall (1943), Sarason (1943) give evidence that there is an intimate relation between the gonads and the adrenal proper, i.e., the cortex apart from the "foetal zone." Gersh and Grollman (1939) and Selye (1950) claim that certain doses of folliculoids have the effect of a nociferous stimulus which evokes from the cortex a non-specific "alarm reaction." In addition, Peczenik (1944) postulates that certain doses of estrogen might act on the adrenal of the golden hamster not only as a nociferous stimulus, but also might provoke a specific hormonal response. That a single injection of stilbestrol is capable of invoking the release of ACTH in the female seems to substantiate the findings of the above investigators.

It has been suggested that ACTH-release is "blocked" in the male but not in the female hamster (Wexler, 1951), and in this present work, that castration removed this "block." This is in agreement with the report of Selye and Stone, 1950, that, in the rat, testosterone is capable of blocking endogenous ACTH-release but not exogenous ACTH.

Peczenik (1944) reports that in the male hamster the adrenal is much heavier than in the female which is the reverse of the situation observed in rats and mice (Bourne and Zukerman, 1940). Peczenik also points out that castrated hamsters have adrenals weighing much less than normal ones. This castration effect is reduced or missing after treatment with male hormone. However, Peczenik finds that in spayed females the adrenal weight is greater than in normal females which is opposite to what has been found in this laboratory, i.e., the adrenal weights of normal females are heavier than those of spayed animals (Table 1). He states further that the adrenal cortices of castrated hamsters are histologically similar to those of non-castrates which have been exposed to cold. These results may be explained if we apply the information gained by the present experiment, i.e., castration permits ACTH-release in the golden hamster. The fact that Peczenik finds that exogenous testosterone prevents these cortical changes adds further support to the supposition that the testes are capable of blocking ACTH-release.

SUMMARY

A single intraperitoneal injection of 0.06 mg. of stilbestrol was given to groups of castrated and spayed hamsters and the animals sacrificed after the expiration of the following time intervals: 0, ½, 1, 3, 6, 12, 24, 36, 48 and 96 hours. Chemical analyses of adrenal glands for ascorbic acid showed that gonadectomy results in an accumulation of adrenal ascorbic acid twice that of the normal value. Also, that castration is associated with the

removal of a purported "blocking agent" to ACTH-release in the male hamster, so that the castrate responds to a single injection of stilbestrol as does the spayed hamster, although at a lower level of ascorbic acid content.

Acknowledgements

We wish to thank Dr. Don Carlos Hines of Eli Lilly and Company for his generous gift of diethylstilbestrol, and Dr. Cathrine Crismon for aid in the statistical analysis of the data.

This investigation was supported in part by research grants from the American Cancer Society, and the National Cancer Institute of the National Institutes of Health, Public Health Service, administered by Dr. Hadley Kirkman.

REFERENCES

BESSEY, O. A.: *J. Biol. Chem.* **126:** 771. 1938.
BOURNE, G. AND S. ZUCKERMAN: *Endocrinology* **2:** 268. 1940.
GERSH, I. AND A. GROLLMAN: *Anat. Rec.* **75:** 131. 1939.
KIRKMAN, H.: (unpublished data). 1951.
KIRKMAN, H. AND R. L. BACON: *Cancer Research* **10:** 122. 1950.
LONG, C. N. H.: *Fed. Proc.* **6:** 461. 1947.
MATTHEWS, V. S., H. KIRKMAN AND R. L. BACON: *Proc. Soc. Exper. Biol. & Med.* **66:** 195. 1947.
PECZENIK, O.: *J. Endocrinology* **3:** 157. 1943.
PECZENIK, O.: *Proc. Roy. Soc. Edinburgh* **62:** 59. 1944.
SARASON, E. L.: *Arch. Path.* **35:** 373. 1943.
SAYERS, G.: *Physiol. Rev.* **30:** 241. 1950.
SCHILLING, W. AND G. LAQUER: *Endocrinology* **29:** 103. 1941.
SELYE, H.: "Stress," ACTA Endocrinologica, Inc., Montreal. 1950.
SELYE, H. AND S. ALBERT: *Proc. Soc. Exper. Biol. & Med.* **50:** 159. 1942.
SELYE, H., E. M. ROWLEY AND C. E. HALL: *Proc. Soc. Exp. Biol. & Med.* **54:** 141. 1943.
SELYE, H. AND H. STONE: "On the Experimental Morphology of the Adrenal Cortex." Charles C Thomas, Publ., Springfield, Ill. 1950.
WEXLER, B. C.: *Endocrinology* **49:** 36. 1951.

THE EFFECT OF CERTAIN ANIONS UPON THE ACCUMULATION AND RETENTION OF IODIDE BY THE THYROID GLAND*

JAMES B. WYNGAARDEN,† BENJAMIN M. WRIGHT‡ AND PETER WAYS‡

From the Thyroid Laboratory of the Massachusetts General Hospital, Boston, Mass.

THE goitrogenic action of thiocyanate has been known since 1936 when Barker reported the occurrence of goiter in patients receiving thiocyanate therapy for hypertension and the correction of such goiter by the administration of desiccated thyroid. Astwood later (1943) produced goiter in rats with thiocyanate, but found no goitrogenic effect when iodide was given simultaneously. The action of thiocyanate was poorly understood until Franklin, Chaikoff and Lerner (1944) demonstrated that it interfered with thyroidal accumulation of iodine, in contrast to thiouracil which blocked the organic incorporation of iodine but did not inhibit its collection by the thyroid. In this way the process of iodine accumulation was clearly distinguished from that of hormone synthesis.

Vanderlaan and Vanderlaan (1947) and Taurog, Chaikoff and Feller (1947) showed that iodine accumulated by animals treated with propylthiouracil was held in the form of the iodide ion. Vanderlaan and Vanderlaan also demonstrated that it could be rapidly and quantitatively flushed from the thyroid by the administration of thiocyanate, but that bromide given in large doses was without effect. Stanley and Astwood (1948) found that thiocyanate and inert iodide discharged radioiodide collected by the thyroid glands of patients treated with antithyroid agents, and more rapidly in thyrotoxic than in normal persons. Stanbury and Hedge (1950) observed a similar discharging action of thiocyanate in certain goitrous cretins who exhibited high radioiodide uptakes.

The present study was undertaken to determine whether or not any

Received for publication November 13, 1951.

* Supported by grants from Parke-Davis and Co., Detroit, Michigan, and from The Atomic Energy Commission, by a grant from The American Cancer Society to the Thyroid Clinic and by an Institutional grant from The American Cancer Society to the Massachusetts General Hospital.

† Present address: Division of Nutrition and Physiology, The Public Health Research Institute of the City of New York, Inc., New York, N.Y.

‡ Present address: College of Physicians and Surgeons, Columbia University, New York, N.Y.

other anions possessed actions similar to those of thiocyanate upon the thyroid. Various anions were assayed for their ability to discharge iodide accumulated within the thyroid, or to block iodide uptake, in the rat treated with propylthiouracil. Anions whose behavior resembled iodide in the Hofmeister series (Glasstone, 1946; Clark, 1948) or in their interaction with serum albumin (Scatchard and Black, 1949) were selected initially, and then the series was extended to include higher or lower oxidation forms of active ions. In addition, certain randomly selected anions were studied. Finally, the goitrogenic action of two members of this series of interfering anions was evaluated.

EXPERIMENTAL

Male Wistar rats weighing 150–175 gm. were fed a low iodine diet (Remington, 1937) containing 0.1% propylthiouracil for 14 or more days. They were then given 10 mg. of propylthiouracil intraperitoneally to insure adequate block. One hour later they were given 5 μg. of sodium iodide^{127} labeled with 10 μc. of sodium iodide^{131}. The thyroidal collection of radioiodide was determined by counting the neck area of the rats every 15 minutes, using a shielded scintillation counter, with sodium iodide crystal, linear amplifier and conventional scaler circuit. The shield consisted of a lead block 5.9 cm. in thickness in the center of which was bored a 2.6 cm. hole. The neck of the rat, or a sealed glass capsule containing the radioiodide standard, when placed against this window, was 20.7 cm. from the counter crystal. The average of three or more 15 second counts was determined, and activity was expressed in per cent of administered dose of iodide. The curves of radioiodide activity reached a maximum 30–45 minutes after the administration of the labeled iodide, following which there was a gradual decline.

The radioactivity arising from other than thyroidal sources under these conditions was determined in rats that had received a dose of thiocyanate sufficient to prevent thyroidal collection of iodide. Labeled iodide was injected 20–60 minutes after the intraperitoneal administration of 10 mg. of potassium thiocyanate. The measurements then obtained indicated that a maximum of 6–9% of the administered radioiodide was detected under these conditions. That these values represented extrathyroidal iodide was proved by the demonstration that thyroids excised from these rats at various times contained almost no radioactivity. The actual uptake of radioiodide by the thyroid was determined from the difference between the total counts measured and the counts recorded at a corresponding time in rats pretreated with thiocyanate.

The experimental compounds were administered intraperitoneally after the collection of radioiodide had been followed for 45 minutes, and further counts were obtained for an additional 75 minutes. Since 10 mg. (approximately 0.1 mM) of potassium thiocyanate had been shown (Vanderlaan and Vanderlaan, 1947) to effect a quantitative discharge of iodide within 15 minutes under these conditions, each compound was administered in an equivalent dose, given as 1 cc. of a 0.1 M solution, and the effect compared with that of thiocyanate. The principal criterion of effectiveness of an experimental compound was taken to be the inducement of a significant loss of counting activity from the region of the thyroid during the first 15 minutes after its administration. The following method was used to express the potency of each compound as a discharger of iodide from the thyroid: the average 45 minute counting value of each group was assigned a value of "no discharge," and the result obtained at a corresponding

time in rats pretreated with thiocyanate was assigned a value of "100% discharge." The effectiveness of a compound was then expressed in terms of the percentage of the collected iodide which it displaced from the thyroid. In 10 control rats during the period from 45 to 60 minutes after the administration of radioiodide the spontaneous discharge never exceeded 25% of that accumulated. In screening an agent, a significant effect was taken to be the discharge of at least 50% of the collected radioiodide during this initial period.

Most of the anions found to discharge trapped iodide were also evaluated for their effect upon the collection of iodide by the thyroid when administered prior to the labeled iodide.

The anions evaluated were iodide, bromide, chloride, fluoride, thiocyanate, periodate,

TABLE 1. GOITROGEN STUDY REGIMENS

Group	Diet	Drinking water	Food intake gm./100 gm. rat/day	Water intake cc./100 gm. rat/day	Drug mg./100 gm. rat/day
I	Regular*	Tap water†	6.8	—	—
II	Regular plus 0.1% propylthiouracil	Tap water	5.5	—	5.5
III	Regular	1.0% KSCN	3.4	10.8	107.5
IV	Regular	1.0% KNO_3	3.6	32.6	326.3
V	Regular	1.0% $KClO_4$	6.3	23.4	233.5

* Purina Fox Chow Diet.
Iodine = 0.32 mg./100 gm.
† Iodine = 3 μg./100 cc.

iodate, biiodate, perchlorate, chlorate, hypochlorite, bromate, nitrate, nitrite, permanganate, persulfate, sulfate, acetate, dibasic phosphate, ascorbate and dehydrocholate. The cation was either sodium or potassium, except that ascorbate was given in the acid form. Three or four rats were used in each experiment. Some rats were returned to the preparatory diet following an experiment, and reused after a minimum of ten additional days, provided that preliminary counting insured the absence of residual radioactivity in the thyroid.

An evaluation of the goitrogenic action of two members of this series was carried out as follows: fifteen rats were divided into five groups of three each and placed on the regimens indicated in Table 1. Frequent determinations of weight and of food, water and drug intakes were made. After 17 days the rats were killed with ether and the weights of their thyroids determined. One-half of each gland was then fixed in formalin for histological sections, which were stained with hematoxylin and eosin. The other halves were pooled by groups and weighed and then homogenized in 1 cc. of cold 10% trichloracetic acid, using an all glass homogenizer. The precipitate was washed with 1 cc. of cold 2.5% trichloracetic acid and again with 5 cc. of distilled water and the supernatants pooled. The precipitate was dissolved in 2 cc. of 2 N sodium hydroxide. Iodine analyses (Barker, 1948, modified) were made to determine the average concentrations of soluble and precipitable thyroidal iodine for each group of rats.

RESULTS

Figure 1 shows the standard rsponses to thiocyanate against which was compared the action of the other ions. Curve A represents the extrathy-

roidal radioiodide detected by the counting system, obtained on rats previously blocked with thiocyanate. Curve B represents the average total activity detected in the necks of 6 rats given radioiodide one hour after propylthiouracil. The gradual decline of curve B represents a shift of equilibrium as iodide is cleared from the plasma by the kidney (Taurog, Chaikoff and Feller, 1947). Curve C demonstrates the effect of 0.1 mM of thiocyanate in promptly discharging the trapped iodide from the thyroid gland. At this dose level of thiocyanate, which exceeds the molar dose of administered iodide by 3,000-fold, the trapped iodide is quantitatively discharged from the thyroid in 15 minutes or less, and thereafter the curve of neck activity coincides with that of extra-thyroidal radioiodine. Thyroid

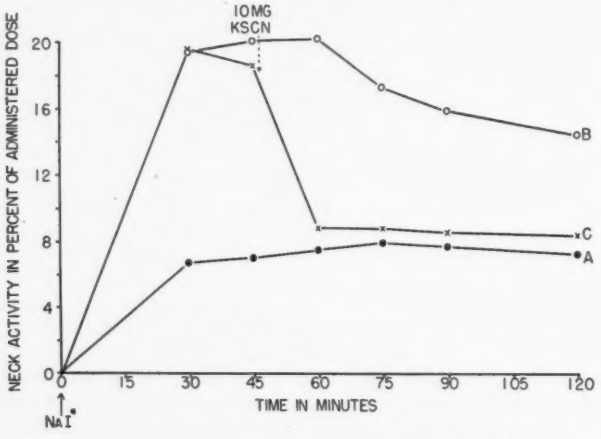

FIG. 1. Effect of pretreatment with thiocyanate upon accumulation of iodide (A) and of subsequent administration upon retention of iodide (C) in the rat treated chronically with propylthiouracil. Curve B is the control group.

glands removed 15 minutes after thiocyanate had been given contained less than 0.8% of the administered dose of labeled iodide, a result in agreement with the findings of Vanderlaan and Vanderlaan (1947).

The administration of the halides fluoride, chloride and bromide in doses of 0.1 mM each had no effect upon iodide accumulated within the thyroid. Administered iodide, however, caused a prompt apparent discharge of radioiodide from the thyroid. This phenomenon probably represents simple dilution of the isotope rather than true iodide discharge.

The halogen oxides proved to be an interesting group. In Figure 2 are shown the effects of these compounds upon iodide accumulated in the blocked gland. Perchlorate, periodate, iodate and chlorate proved capable of causing a quantitative discharge of iodide, although only perchlorate

effected a complete discharge in the initial 15 minute period. Biiodate and hypochlorite in the doses employed caused an incomplete discharge and bromate was negative.

Of the other anions only nitrate proved to be effective and then only weakly so. The results with nitrite were equivocal in that the initial discharge was not significant but the subsequent loss of radioiodide exceeded the spontaneous loss in the control groups. Permanganate, persulfate, sulfate, dibasic phosphate, acetate, ascorbate and dehydrocholate were ineffective.

In order to establish the relative potencies of perchlorate, thiocyanate

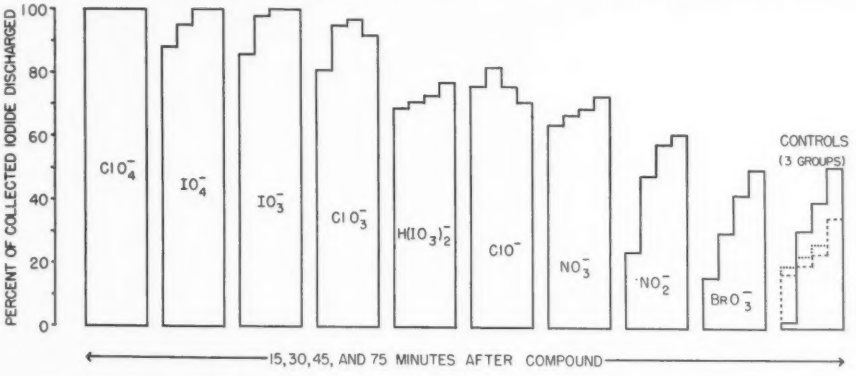

FIG. 2. Effect of 0.1 millimole of certain anions upon the retention of iodide in the thyroid of rats chronically treated with propylthiouracil. Data plotted represent discharged iodide expressed cumulatively.

and nitrate as discharging agents, these anions were assayed in consecutive logarithmic reductions of dosage, starting with a dose of 10^{-4} M of each agent. These three anions were selected since they spanned the group in range of activity. The results of this dose-response study are given in Figure 3. It is seen that a dose of 10^{-7} M of perchlorate is capable of discharging 50% of the newly collected iodide. This dose of perchlorate exceeds the dose of iodide administered (3.3×10^{-8} M) by only 3-fold. However, only 13.3% of the iodide had been collected by the thyroid, so that the true ratio of perchlorate administered to iodide discharged may be as high as 23 to 1. A dose of thiocyanate approximately 10 times and of nitrate approximately 300 times that of perchlorate was required to effect the same result.

In Figure 4 the effect of 0.1 mM of several of these agents upon subsequent iodide accumulation is shown. At this dose level the degree of block effected varied considerably among the different anions. Perchlorate proved

to be the most potent anion of the experimental group in this study also.

Figure 5 represents a study of competition between perchlorate and iodide. A dose of perchlorate known to be incompletely effective in discharging iodide from the thyroid was given to four rats. Twenty minutes later, two rats were given 5 μg. and two 100 μg. of labeled iodide. The total amount of iodide trapped in the latter instance is about 6 times greater than in the former, although the percentage of the administered dose collected is smaller. This finding suggests that large doses of iodide may nullify the effects of perchlorate just as they do the effects of thiocyanate.

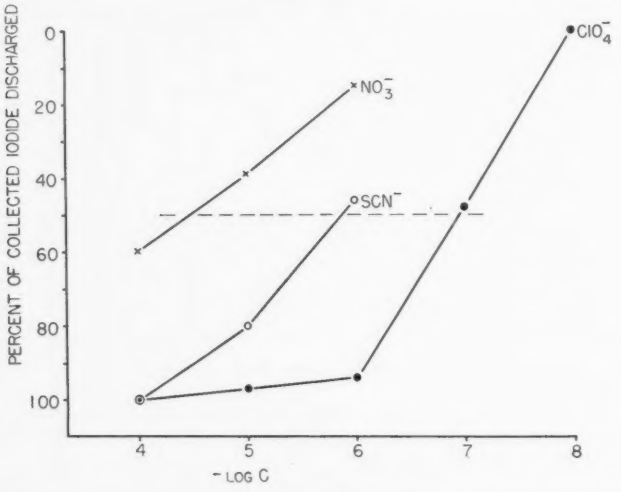

FIG. 3. Dose-response data for the effects of three anions in discharging iodide from the thyroid gland.

In the evaluation of the goitrogenic effects of dietary perchlorate and nitrate, these anions were compared with thiocyanate and propylthiouracil with respect to influence upon body weight and upon thyroidal weight, iodine concentrations and histologic pattern. After 17 days the rats treated with perchlorate and nitrate had gained as much weight as had the controls, whereas the rats treated with thiocyanate and propylthiouracil showed retardation of growth (Figure 6). In Table 2 are given data on weights of thyroids, analyses of tissue iodine concentrations and degrees of hyperplasia in the five rat groups. Photomicrographs of hyperplastic tissue resulting from perchlorate and nitrate administration are presented in Figure 7 and compared with histologic patterns of thyroids from rats of the other groups.

It is apparent that rats tolerate large doses of perchlorate and nitrate well. Perchlorate proved to be a very effective goitrogen, leading to marked hypertrophy and hyperplasia, to a great increase in vascularity of thyroid tissue, and to profound reductions of both soluble and precipitable iodine. In these respects it proved fully as effective as propylthiouracil and more so than thiocyanate. Nitrate produced only a minimal hypertrophy and hyperplasia and a slight reduction of iodine concentrations.

We did not determine the effectiveness of other interfering anions as

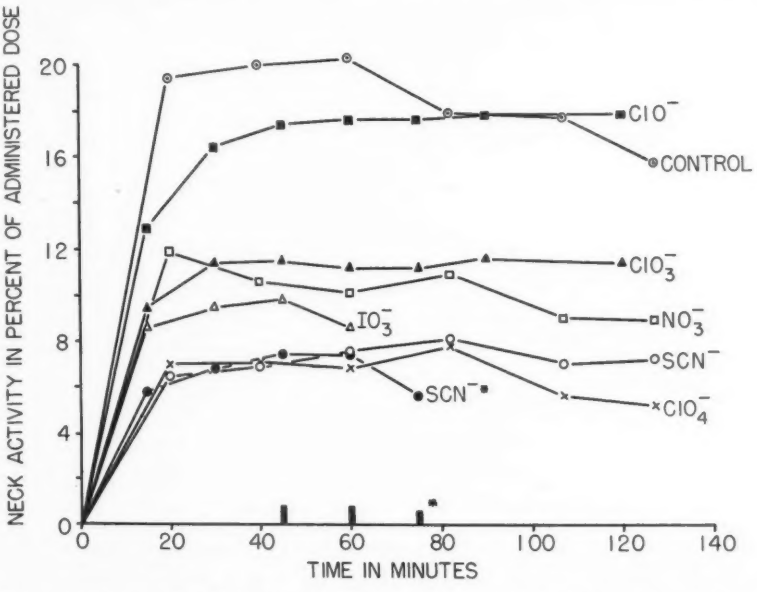

FIG. 4. Effect of administration of 0.1 millimole of certain anions upon subsequent collection of iodide by the thyroid. Anions were administered 20 minutes prior to the labeled iodide. The three bar graphs represent actual thyroidal collection of iodide following the administration of thiocyanate in the group marked by the asterisk, at the indicated times.

goitrogens, but the toxicity of the various iodates would probably preclude their use. In the studies described in this paper all rats given periodate, iodate, biiodate, bromate and permanganate died within 3–16 hours.

DISCUSSION

An understanding of the mode of action of these anions upon the mechanism of iodine collection by the thyroid must await a better definition of that process itself, for the mechanism by which the thyroid is able to ac-

cumulate inorganic iodine, and to maintain concentration gradients of 25 to 500-fold over the serum under various conditions (Vanderlaan and Vanderlaan, 1947; Taurog, Chaikoff and Feller, 1947; Taurog, Tong and Chaikoff, 1951) is as yet unknown. The iodine accumulated by this process in the animal treated with propylthiouracil is bound very loosely by the thyroid tissue, is dialyzable, ultrafiltrable and nonprecipitable and is held in the form of the iodide ion (Vanderlaan and Vanderlaan, 1947; Taurog,

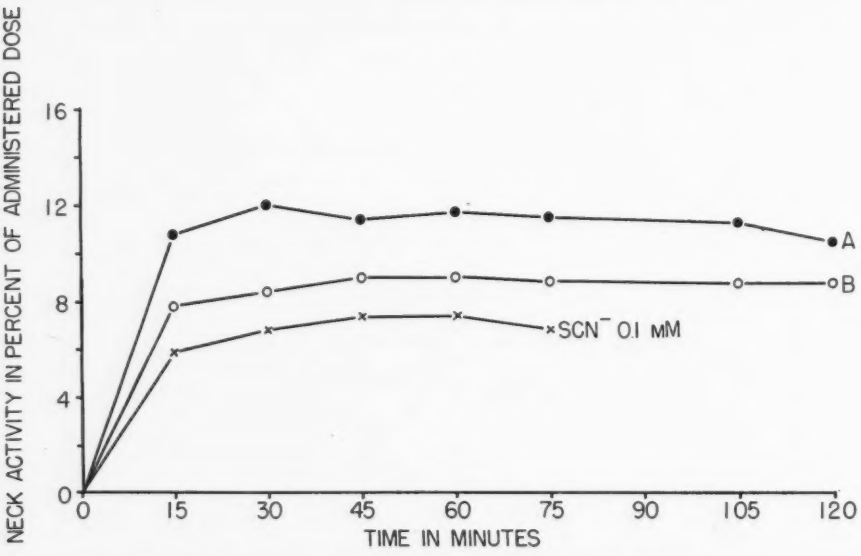

FIG. 5. Effect of pretreatment with 0.001 millimole of perchlorate upon subsequent collection of iodide by the thyroid when a small and a large dose of iodide were given. The rats of curve A received 5 μg. of NaI, and those of curve B, 100 μg. The maximal accumulation of iodide in the former group is 5.2% of the administered dose at 30 minutes and of the latter 1.6% at 45 minutes, or 0.26 μg. and 1.6 μg. of iodide, respectively.

Chaikoff and Feller, 1947). It is this iodide whose collection or retention is inhibited by thiocyanate and by the anions described in the present study.

Vanderlaan and Greer (1950) have shown that the activity of the iodide concentrating mechanism of the thyroid gland is strikingly diminished in the absence of pituitary thyrotropin. This suggests two possible sites of action of the thiocyanate-perchlorate ion group: These ions could act to place a block between thyrotropin and the enzyme system of the thyroid responsible for iodide collection, or they could act to inhibit the enzyme system directly. The rapidity with which they act would favor the latter interpretation, while the effect of an ion such as perchlorate on histologic

TABLE 2. GOITROGEN STUDY RESULTS

Group	Rat	Drug	Thyroid weight, mg.	Average weight, mg.	Degree of microscopic hyperplasia*	Iodine in μg./mg. tissue	
						Soluble†	Precipitable
I	A	None	6.0	11.4	+	0.099	0.700
	B		16.4		+		
	C		11.9		0		
II	A	Propylthiouracil	22.9	33.7	+++	0.019	0.023
	B		42.8		+++		
	C		35.4		+++		
III	A	KSCN	37.4	29.7	++	0.023	0.091
	B		28.2		++		
	C		23.4		++		
IV	A	KNO_3	16.6	14.9	++	0.088	0.536
	B		14.7		++		
	C		13.4		+		
V	A	$KClO_4$	32.0	31.9	++++	0.018	0.021
	B		27.0		+++		
	C		36.7		++++		

* We are indebted to Dr. Auston Vickery for the histological grading of the tissues which were presented to him as unknowns.

† Taurog, Tong and Chaikoff (1951) have recently shown that inorganic iodide comprises only 25 to 50% of the iodine in the trichloracetic acid extract of the thyroid, and that the remainder consists of thyroglobulin and a small amount of diiodotyrosine.

pattern, colloid storage and iodine concentrations of the thyroid would indicate that their regulation by thyrotropin (Vanderlaan and Greer, 1950; Goldsmith, Stanbury and Brownell, 1951) is not impaired by the adminis-

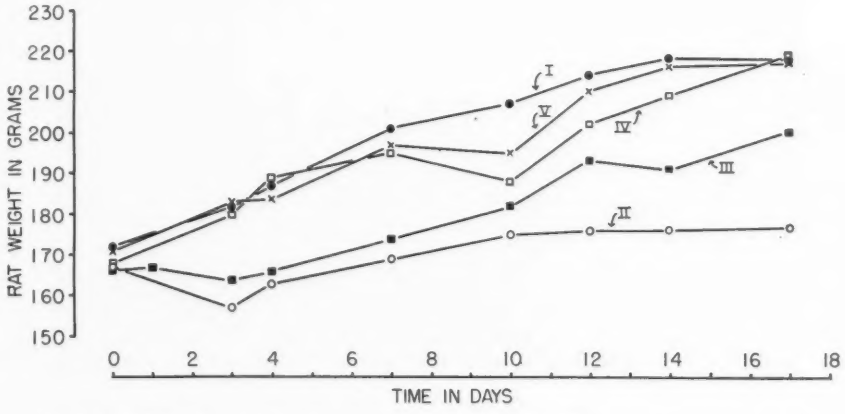

FIG. 6. Effect of certain goitrogens upon rat growth. The roman numeral labels correspond to the 5 groups as defined in Tables 1 and 2.

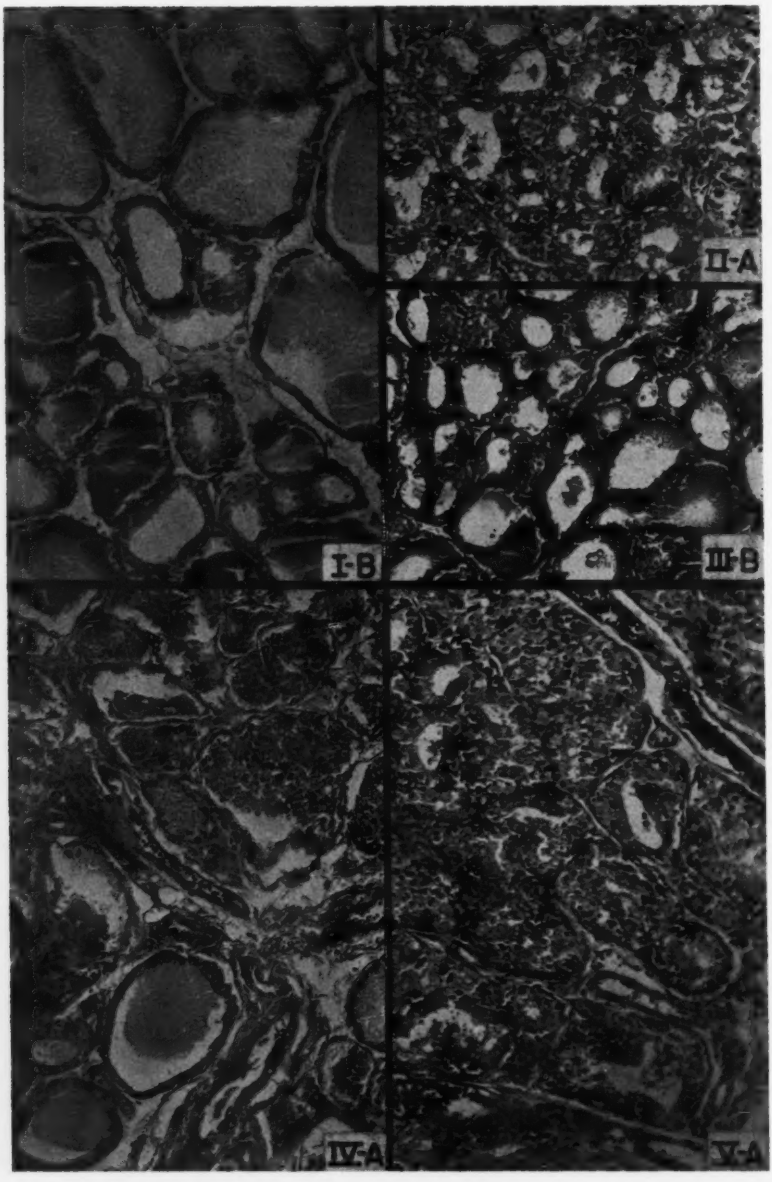

FIG. 7. Photomicrographs of thyroid glands excised from rats after the study described in Tables 1 and 2. The labels refer to specific animals as designated in Table 2. 1B—normal control. 2A—Propylthiouracil. 3B—Thiocyanate. 4A—Nitrate. 5A—Perchlorate. (125 X)

tration of perchlorate. And while it is possible that only the control of iodide collection by thyrotropin is blocked by these anions, there is no basis at present for such a contention.

A third possible mode of action of these agents is that of competition with iodide for collection by the thyroid itself. In this connection Baumann and Metzger (1949) have postulated that the thyroid concentrates all halides with imperfect distinction and that thiocyanate behaves as a halide. The evidence for this thesis is not impressive, for whereas an avid collection of eka-iodine (Hamilton and Soley, 1940) and a slight accumulation of bromide (Perlman, Morton and Chaikoff, 1941) within the thyroid have been demonstrated, there is no proof that fluoride, chloride or thiocyanate is accumulated by the gland.

In the present study, massive doses of fluoride, chloride and bromide failed to effect any discharge of thyroidal iodide. Also, Williams, Jaffe and Taylor (1950) have recently found that chloride and bromide do not interfere with collection of iodine by the thyroid. The augmentation of the goitrogenic effects of small doses of thiouracil by these anions and by fluoride is probably due to a competitive interference with the readsorption of iodide in the renal tubule (Williams, Jaffe and Soloman, 1950). The maximal recorded levels of about 6 meq. of chloride per 100 gm. of thyroid tissue and the maximal thyroid-*blood* ratios of thiocyanate of two (Baumann and Metzger, 1949), do not suggest that thyroid-*serum* gradients are present for these ions. When tracer doses of labeled thiocyanate are given to rats, and isotopic *thiocyanate-ion* sulfur is specifically determined, no thyroidal collection is found (Wood and Kingland, 1950) although *total* isotopic sulfur may be concentrated as much as 8-fold, (Wood and Williams, 1949; Stanbury, 1951). Certainly the uptakes of chloride and thiocyanate do not resemble thyroidal concentration of iodide in kind or magnitude.

Despite the lack of precedent for thyroidal collection of anions other than iodide, eka-iodide and bromide, it would appear worthwhile to investigate accumulation of the group presented, if only to rule out such a phenomenon. In the only such study of a member of this ion series, other than of thiocyanate itself, Leblond and Süe (1941) found that iodate was not concentrated by the thyroid of the normal rat. However, perchlorate apparently is concentrated within certain endocrine organs (Durand, 1938), although no data relating directly to the thyroid are available. Even if no collection of these inhibiting anions occurs within the thyroid gland, it is conceivable that they perform in the role of substrates competing for the enzyme system normally employed in iodide accumulation. In this connection, the metabolism of thiocyanate by the thyroid may be recalled (Wood and Williams, 1949).

The anions found to be effective in this study are in many cases situated

close to iodide in the Hofmeister series, and have a resemblance to iodide in the nature of their ionic interaction with pure serum albumin. The singular failure among the halogen oxides of bromate to exert any effect is perplexing. These facts may become of importance in understanding the mode of action of these agents upon the thyroid. At present, it must suffice simply to state that these anions act to destroy, with varying degrees of effectiveness, the iodide concentrating capacity of the thyroid gland, probably through some influence, as yet obscure, upon the responsible enzyme system.

SUMMARY

The monovalent anions perchlorate, chlorate, hypochlorite, periodate, iodate, biiodate and nitrate have been found to share with thiocyanate the properties of inhibiting collection and of interfering with retention of the iodide ion within the thyroid glands of rats chronically treated with propylthiouracil. A dose response study revealed perchlorate to be 10 times and nitrate 1/30 as potent as thiocyanate in discharging iodide previously collected by the thyroid; the other anions appeared to occupy positions of intermediate potency. The capacity of these agents to prevent the collection of iodide by the thyroid approximately paralleled their iodide discharging action. Rats treated with perchlorate for 17 days developed hyperplastic, colloid-depleted, low-iodine goiters. These changes were as marked as those resulting from propylthiouracil ingestion. In contrast, rats treated with nitrate developed only minor degrees of hyperplasia and a slight reduction of thyroidal iodine concentrations. Possible mechanisms of action of this group of anions are discussed.

Acknowledgments

The authors are indebted to Drs. J. H. Means and John B. Stanbury for the opportunity to pursue this study, and to Misses Helen Hagopian and Betty Rapp for able technical assistance during a part of it.

REFERENCES

ASTWOOD, E. B.: *J. Pharmacol. and Exper. Therap.* **78**: 79. 1943.

BARKER, M. H.: *J.A.M.A.* **106**: 762. 1936.

BARKER, S. B.: *J. Biol. Chem.* **173**: 715. 1948.

BAUMAN, E. J., AND N. METZGER: *Proc. Soc. Exper. Biol. and Med.* **70**: 563. 1949.

CLARK, W. M.: Topics in Physical Chemistry. The Williams and Wilkins Co., Baltimore. 1948. p. 681.

DURAND, J.: *Bull. Soc. Chim., biol.* **20**: 423. 1938.

FRANKLIN, A. L., I. L. CHAIKOFF AND S. R. LERNER: *J. Biol. Chem.* **153**: 151. 1944.

GLASSTONE, S.: Textbook of Physical Chemistry. J. Van Nostrand Co., Inc. New York, 2nd Edition. 1946. p. 1254.

GOLDSMITH, R. E., J. B. STANBURY AND G. L. BROWNELL: *J. Clin. Endocrinol.* **11**: 1079. 1951.

HAMILTON, J. G. AND M. H. SOLEY: *Proc. Nat. Acad. Sci.* **26**: 483. 1940.
LEBLOND, C. P. AND P. SŪE: *Am. J. Physiol.* **134**: 549. 1941.
PERLMAN, I., M. E. MORTON AND I. L. CHAIKOFF: *J. Physiol.* **134**: 107. 1941.
REMINGTON, R. E.: *J. Nutrition* **13**: 223. 1937.
SCATCHARD, G. AND E. S. BLACK: *J. Physical and Colloid Chem.* **53**: 88. 1949.
STANBURY, J. B. AND A. HEDGE: *J. Clin. Endocrinol.* **10**: 1471. 1950.
STANBURY, J. B.: Unpublished data. 1951.
STANLEY, M. M. AND E. B. ASTWOOD: *Endocrinology* **42**: 107. 1948.
TAUROG, A., I. L. CHAIKOFF AND D. D. FELLER: *J. Biol. Chem.* **171**: 189. 1947.
TAUROG, A., W. TONG AND I. L. CHAIKOFF: *J. Biol. Chem.* **191**: 677. 1951.
VANDERLAAN, J. E. AND W. P. VANDERLAAN: *Endocrinology* **40**: 403. 1947.
VANDERLAAN, W. P. AND M. A. GREER: *Endocrinology* **47**: 36. 1950.
WOOD, J. L. AND N. KINGSLAND: *J. Biol. Chem.* **185**: 833. 1950.
WOOD, J. L. AND E. F. WILLIAMS, JR.: *J. Biol. Chem.* **177**: 59. 1949.
WILLIAMS, R. H., H. JAFFE AND B. SOLOMAN: *Am. J. Med. Sc.* **219**: 1. 1950.
WILLIAMS, R. H., H. JAFFE AND J. A. TAYLOR: *Am. J. Med. Sc.* **219**: 7. 1950.

THYROID RESPONSE TO TOTAL BODY X-IRRADIATION

A. L. BOTKIN, E. H. PRAYTOR, M. E. AUSTING AND H. JENSEN

From the Army Medical Research Laboratory, Fort Knox, Kentucky

DURING the past several years there have been many investigations into the effects of either total body or local x-irradiation on various mammalian organs. In the case of the thyroid gland, however, practically all of the studies undertaken have dealt with direct irradiation of the gland by local x-irradiation (Bender, 1948; Hursh *et al.*, 1949) or by massive doses of radioactive iodine (Goldberg *et al.*, 1950; Gorbman, 1950). These studies have shown that in the rat local irradiation of the thyroid produces essentially no change in morphology or function of that gland until the dosage reaches 3000–6000 r, a dose which is several times the magnitude of a lethal dose of total body x-irradiation.

In a brief abstract, Evans *et al.* (1949), reported an increased uptake of I^{131} by the thyroid as well as "increased activity" in the blood of rats three days after 500–1000 r total body x-irradiation. However, the reported data are rather incomplete and do not allow any definite conclusions as to the state of functional activity of the thyroid after total body x-irradiation.

It was the purpose of the present investigation to determine the content of radioactive iodine (I^{131}), both total and organic, in the thyroid gland as well as the total and protein-bound I^{131} content of blood of rats at various time intervals after a potentially lethal dose (1000 r) of x-radiation. It is felt that such data might indicate any possible changes in thyroid activity during the post-radiation period and thus present a possible aid in the interpretation of the physiological aberrations following lethal radiation.

MATERIALS AND METHODS

Male rats of the Sprague-Dawley strain (weighing 190–250 grams) were used. They were maintained on Purina laboratory chow and tap water until 24 hours before sacrifice, at which time they were deprived of food but allowed water *ad lib.*

The rats were irradiated, two at a time, in a well-ventilated lucite chamber. The radiation was performed with a 250 Kv Kelly-Koett x-ray unit, the factors being: 200 Kv., 6 ma., ½ mm. copper and 1 mm. aluminum filters, target distance 29 cm. This set-up gave a dosage of 40 roentgens per minute as measured in air with a Victoreen

Received for publication December 5, 1951.

thimble chamber.[1] Each rat received 1000 r (25.0 min.) which was found to correspond to an LD/100 (8 days), with the maximal mortality occurring between the third and fifth day.

The animals under light nembutal or ether anesthesia were sacrificed by exsanguination via cardiac puncture at "zero," 2 hours, 1 day, 2 days, 3 days, 4 days and 6 days after irradiation. Twenty-four hours before sacrifice each rat was injected intraperitoneally with 1.0 ml. of a standardized radioactive iodine solution containing approximately 5.0 microcuries of carrier-free radioiodine (I^{131})[2] made to volume with Krebs-Henseleit buffer of pH 7.4 (Krebs and Henseleit, 1932).

After sacrifice, the thyroid gland was rapidly removed, weighed, and placed in hot 2 N NaOH for hydrolysis. Adrenal glands were removed and weighed at the same time.

The thyroid glands were processed and analyzed for their total and organic bound radioiodine contents and the serum analyzed for its total and protein-bound (PBI) radioiodine contents using the methods of Morton and Chaikoff (1943). Control groups of 6 rats were run with each set of irradiated animals. Groups of 8 to 24 experimental animals were used for each time period.

All values for radioiodine content were calculated as per cent of the injected dose (Table 1).

TABLE 1. THYROID GLAND AND SERUM RADIOIODINE CONTENTS AS MEAN PER CENT OF THE INJECTED DOSE WITH STANDARD ERROR*

Time after irradiation	Thyroid gland				Blood serum			
	Control		Experimental		Control		Experimental	
	Total	Organic	Total	Organic	Total	PBI	Total	PBI
"Zero"	24.7±4.7	16.6±2.6	22.5±3.4	15.4±2.3	9.56±2.02	1.02±0.45	7.98±1.34	1.09±0.26
2 Hours	19.8±1.6	14.2±1.9	18.5±2.2	15.6±1.8	8.38±1.20	1.34±0.73	7.29±1.11	1.77±0.29
1 Day	17.9±2.6	14.0±1.2	17.8±2.1	13.8±1.9	8.07±1.21	0.78±0.19	13.16±1.64	1.11±0.22
2 Days	23.0±5.3	17.6±2.1	18.1±1.1	13.3±2.8	14.96±3.42	4.18±1.71	6.48±1.00	1.38±0.37
3 Days	17.0±2.4	13.6±2.3	12.1±1.0	9.9±0.7	12.84±3.26	1.70±0.65	10.56±2.08	0.65±0.16
4 Days	17.4±2.1	14.3±1.6	5.5±2.3	4.7±1.8	11.30±1.14	1.73±0.60	8.48±1.79	0.47±0.11
6 Days	15.4±2.5	10.9±2.4	9.1±2.7	8.0±2.3	16.31±2.97	1.92±0.33	27.97±1.70	0.15±0.12

* Computed from "t" values for a 90% Fiduciary limit.

RESULTS

It can be seen from Table 1 that as early as 2 hours after irradiation, there is a rise in organic gland I^{131} content (with a resulting rise in the organic/total ratio), accompanied by a similar picture in the serum total and PBI^{131} fractions. By 24 hours the gland content is back to normal while there is a rise in both the serum total and PBI^{131}. Between the first and fourth day after irradiation the gland content, showing a remarkably constant organic/total ratio, drops steadily below control values. By the sixth day there is a slight rise, but the gland content is still below normal. The serum I^{131} content, both total and PBI, drops below control values between

[1] The authors wish to express their appreciation to the Radiobiology Branch of this laboratory for assistance in the irradiation procedure.

[2] The radioactive iodine (I^{131}) used in this investigation was supplied by the Oak Ridge National Laboratories on allocation from the Isotopes Division, U. S. Atomic Energy Commission.

the first and second day after irradiation. From the second to the sixth post-irradiation day, the total serum I^{131} content rises steadily above control values while the serum PBI^{131} falls progressively below normal.

DISCUSSION

Since it has been shown (Bender, 1948; Hursh *et al.*, 1949; Goldberg *et al.*, 1950; Gorbman, 1950) that local irradiation of the thyroid produces no noticeable change in morphology or function of that endocrine organ until the dosage reaches 3000–6000 r, the response of the thyroid gland to 1000 r total body x-irradiation is probably brought about by some systemic disturbance, which may be mediated through the hypophysis.

The increase in serum PBI^{131} as early as 2 hours and as late as one day after irradiation probably indicates stimulation of the thyroid. The slope of the curve as well as the time of response is quite similar to that following a single injection of thyrotropic hormone (Botkin and Jensen, 1952). This thyroid response agrees with the finding of Kirschner *et al.* (1949) that there is an elevation of oxygen consumption of 35% occurring in rats within 24 hours after total-body irradiation of 809–972 r.

From the second until the sixth post-irradiation day, both the gland and serum I^{131} criteria responses are indicative of decreased thyroid activity, giving a picture similar to that observed in hypophysectomized animals (Ghosh *et al.*, 1951; Albert and Lorenz, 1951).

In an investigation of the adrenal response to total body x-irradiation of comparable magnitude to that used in the present studies, Patt and associates (1947) found that there is an initial stimulation of the adrenal (3 to 6 hours after irradiation), followed by an apparent return to normal until after the second post-irradiation day. Thenceforth until death there appears to be a steady increase of adrenal size and function. The above authors point out the similarity of the early adrenal response to that observed following an injection of the adrenocorticotropic hormone.

Adrenal weights taken in the present studies are quite similar to those reported by Patt and his associates (1947).

A comparison of the results presented with those of the adrenal studies described above, is illustrated in Figure 1. These findings may be interpreted as follows: Systemic disturbance due to total body x-irradiation, causes an intial increased elaboration of thyrotropic and adrenocorticotropic principles from the anterior pituitary, followed by a continuous increased demand for adrenal cortical hormones which can only be met by increased pituitary release of adrenocorticotropin at the expense of thyrotropin production. It should be emphasized, however, that there may be other possible interpretations of the present findings.

It is known that the food intake of irradiated animals is less than normal

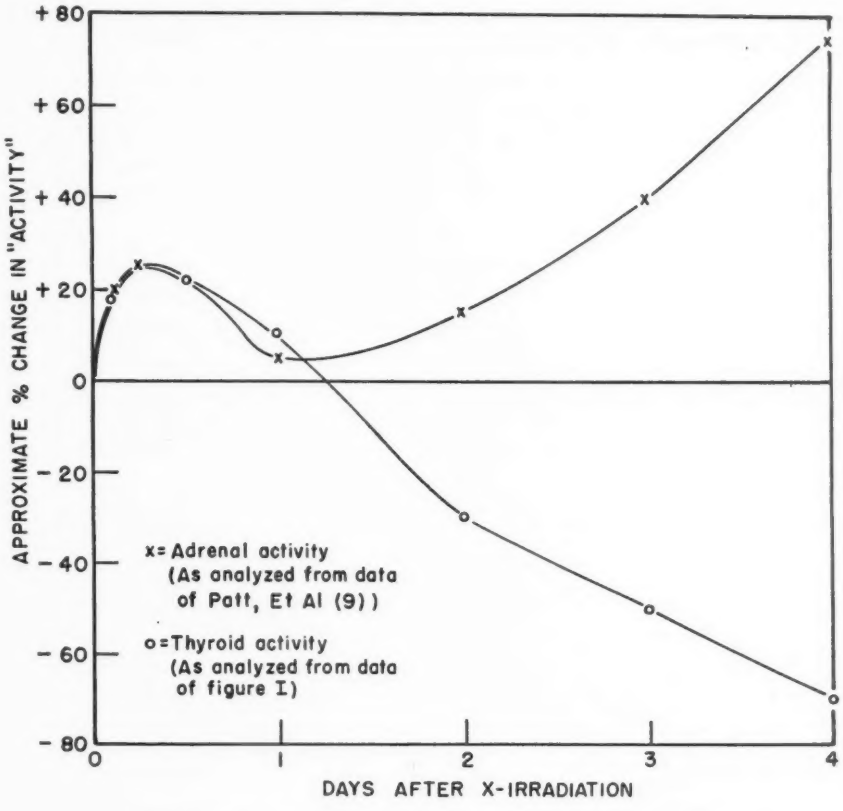

FIG. 1. Thyroid and adrenal activities at intervals after total body x-irradiation (1000 r).

and it has been reported that inanition will cause a shift of pituitary function towards increased adrenocorticotropin elaboration at the expense of thyrotropin production (White and Dougherty, 1947; D'Angelo, 1951).

Thyroid studies done in this laboratory on starved normal rats show a serum I^{131} distribution picture similar to that shown in Table 1, but much smaller in magnitude and not apparent until after the third day of starvation. However, there is no alteration in thyroid I^{131} content by the fourth day. The partial inanition caused by radiation would seem, then, to account for only part of the observed changes.

SUMMARY

The changes in thyroid and serum I^{131} content (inorganic and organic)

of rats, after total body x-irradiation at 1000 r (LD-100/6–8 days), indicate a stimulation of thyroid activity within two hours after irradiation. This increased activity is apparent for 24 hours after irradiation, after which time until the sixth day there is a progressive decrease in activity. These alterations in functional activity of the thyroid may possibly be due to systemic changes caused by the radiation and are probably mediated through the hypophysis. Possible interpretations of the findings are discussed.

REFERENCES

ALBERT, A. AND N. LORENZ: *Proc. Soc. Exper. Biol. and Med.* **77:** 204. 1951.

BENDER, A. E.: *Brit. J. Radiol.* **21:** 244. 1948.

BOTKIN, A. L. AND H. JENSEN: *Endocrinology* **50:** 68. 1952.

D'ANGELO, S. A.: *Endocrinology* **48:** 341. 1951.

EVANS, T. C., G. CLARKE AND E. SOBEL: *Anat. Rec.* **99:** 577. 1949.

GHOSH, B. V., D. M. WOODBURY AND G. SAYERS: *Endocrinology* **48:** 631. 1951.

GOLDBERG, R. C., I. L. CHAIKOFF, S. LINDSAY AND D. D. FELLER: *Endocrinology* **46:** 72. 1950.

GORBMAN, A.: *Tran. N. Y. Acad. Sci.* **11:** 201. 1949.

HURSH, J. B., J. B. MAHONEY AND P. A. VAN VOLKENBURG: *U. S. Atomic Energy Commission, Technical Information Division, Oak Ridge, Tennessee* AECU-615 (UR-89), 1949.

KIRSCHNER, L. B., C. L. PROSSER AND H. QUASTLER: *Proc. Soc. Exper. Biol. and Med.* **71:** 463. 1949.

KREBS, H. A. AND K. HENSELEIT: *Hoppe-Seyler's Ztschr. f. physiol. Chem.* **210:** 33. 1932.

MORTON, M. E., AND I. L. CHAIKOFF: *J. Biol. Chem.* **147:** 1. 1943.

PATT, H. M., M. N. SWIFT, E. B. TYREE AND E. S. JOHN: *Am. J. Physiol.* **150:** 480. 1947.

WHITE, A. AND T. F. DOUGHERTY: *Endocrinology* **48:** 230. 1947.

FAILURE OF ADRENOCORTICOTROPIN AND VARIOUS STIMULI TO DEPLETE THE ASCORBIC ACID CONTENT OF THE ADRENAL GLAND OF THE QUAIL[1]

M. X. ZARROW AND J. T. BALDINI[2]

From the Departments of Biological Sciences and Poultry Husbandry, Purdue University, Lafayette, Indiana

WITHIN recent years a number of procedures have been devised to determine the secretory activity of the adrenal cortex. One of the tests most commonly used is the loss of ascorbic acid from the adrenal gland. The depletion of the adrenal gland of its ascorbic acid and cholesterol following the administration of adrenocorticotropin (ACTH) has been amply confirmed by many investigators since it was first reported by Sayers, Sayers, Fry, White and Long (1944) and Sayers, Sayers, Liang and Long (1947). These investigators reported a maximum drop in the adrenal ascorbic acid within one hour and in the adrenal cholesterol within four hours after the injection of ACTH. Exposure of normal animals to such stressful stimuli as cold, scalding, formaldehyde injections etc. likewise produced a decrease in both adrenal ascorbic acid and cholesterol. As these changes did not occur in hypophysectomized animals it was concluded that the effect was mediated through the release of ACTH from the pituitary gland. Much use has been made of this reaction and in a recent review, Sayers (1950) has admirably summarized the vast amount of data that has been reported in the last few years on the pituitary-adrenal physiology.

The above reaction has also been used for the assay of ACTH in hypophysectomized rats (Sayers, Sayers, and Woodbury, 1949). It has been shown that a linear relationship exists between the loss of adrenal ascorbic acid and the amount of ACTH injected. In an attempt to utilize the normal chick as a test animal, Jailer and Boas (1950) noted that neither ACTH nor epinephrine cause a loss in adrenal ascorbic acid. This has been confirmed in the duck by Zarrow and Zarrow (1950).

Thus far the depletion of the adrenal ascorbic acid following treatment with ACTH has been described in the rat, mouse, and guinea pig and has

---

Received for publication December 14, 1951.

[1] Journal Paper No. 588 of the Purdue University Agr. Exp. Sta.

[2] Present address: E. I. duPont de Nemours and Co., New Brunswick, New Jersey.

led investigators to assume a relationship between ascorbic acid and the synthesis or release of adrenal corticoids. It is evident that this relationship does not hold for all species and certainly not for the hen or duck in which the adrenal ascorbic is not affected by ACTH. This would tend to indicate that birds do not react in a manner similar to that noted for rats and would cast some doubt as to the essential role of ascorbic acid in the synthesis and release of adrenal corticoids. Before such a generalization can be made it was felt necessary to examine the phenomenon in another avian species. This investigation was undertaken to study the adrenal ascorbic acid in a non-domesticated bird, the bobwhite quail (*Colinus v. virginianus*).

MATERIAL AND METHODS

Approximately one hundred bobwhite quail (*Colinus v. virginianus*)[3] were used in this study. The birds were hatched in the spring and kept in batteries under standard laboratory conditions. Food and water was supplied *ad libidum*. The birds showed excellent growth (Baldini, 1951) and appeared in good condition throughout the course of this work. Studies were carried out on both males and females varying in age from 8 to 18 weeks.

The experimental birds were injected intraperitoneally with ACTH[4] and the ascorbic acid concentration of the adrenal gland determined 1 hour later. In addition, cold (2 to 4° C.) and epinephrine (intramuscular injection of 0.05 ml. of a 1:1000 solution) were used as stressfull stimuli in the short term studies. In a second experiment, two groups of quail were injected with ACTH for 7 and 14 days respectively. The ACTH was dissolved in saline and injected intramuscularly, alternating the site of injection between the pectoralis and the femoral muscles. In a limited number of birds the effect of estrogens on adrenal size was examined. These quail were treated daily with intramuscular injections of estradiol in oil or with the implantation of pellets of stilboestrol.

All birds were killed by decapitation and the adrenals rapidly removed and weighed on a Roller-Smith torsion balance. The adrenal glands from two quail were pooled and the ascorbic acid concentration determined by the method of Roe and Keuther (1943) using a Klett photoelectric colorimeter.

RESULTS

The adrenal gland of the bobwhite quail was found to contain approximately 150 mg. per cent ascorbic acid and the concentration did not change with the age of the birds used in this study, i.e. from 8 weeks of age to maturity. Furthermore no sex differences in the ascorbic acid content of the adrenal glands were discernible in any of the age groups (Table 1). Although the adrenal glands of the female quail (8–12 weeks of age) weighed slightly more than the adrenals of the male birds, this was

[3] The quail were obtained through the courtesy of the Indiana State Conservation Department.

[4] The ACTH was obtained through the courtesy of Dr. Leighty, Eli Lilly and Co., Indianapolis.

not found to be statistically significant. In the mature quail, both sexes possessed adrenals of essentially similar weight. In the adult male the adrenals weighed 16.9 mg. or 9.4 mg. per cent body weight in the females 17.3 mg. or 9.1 mg. per cent body weight.

TABLE 1. EFFECT OF AGE AND SEX ON THE ASCORBIC ACID CONTENT OF THE ADRENAL GLAND

No. of quail	Age weeks	Sex	Body wt., gm.	Adrenal gland		
				Wt., mg.	Body wt., mg. %	Ascorbic acid, mg. %
10	8 to 9	M	114	11.8	10.3	145
9	8 to 9	F	116	13.3	11.4	144
9	12	M	145	13.5	9.5	154
10	12	F	141	15.7	11.2	157
6	Adult	M	178	16.9	9.4	145
7	Adult	F	189	17.3	9.1	156
						150 Average

The effect of various stimuli on the adrenal ascorbic acid was examined in a number of birds varying from 9 to 12 weeks of age (Fig. 1). A single subcutaneous or intraperitoneal injection of 4 to 10 mg. of ACTH failed to deplete the adrenal ascorbic acid within one hour after the injection. The adrenal glands of the control birds and the birds treated with 4 mg. of ACTH contained 145 mg. per cent ascorbic acid and those treated with 10 mg. of ACTH contained 150 mg. per cent ascorbic acid. In several control tests it was noted that 0.5 mg. of the ACTH preparation was more than sufficient to produce a maximum depletion in the adrenal ascorbic acid of the hypophysectomized rat. Three groups of quail were exposed to a temperature of 2 to 4° C. for 1, 2, and 3 hours and showed adrenal ascorbic acid concentrations of 158, 166, and 164 mg. per cent respectively. Finally injections of epinephrine also failed to deplete the adrenal ascorbic acid (Fig. 1).

In an attempt to determine whether ACTH could cause hypertrophy of the adrenal gland of the quail the hormone was injected for 7 and 14 days. In the first experiment 1 mg. of ACTH was injected daily by the intramuscular route for 14 days and the quail killed on the 15th day. All the treated birds lost weight during the course of the treatment (an average loss of 17 gm.), while the untreated control birds showed no appreciable weight change. The adrenal glands of the control quail weighed 17.1 mg. or 9.0 mg. per cent body weight while the ACTH, treated birds had adrenals weighing 21.5 mg. or 13.1 mg. per cent body weight. Analysis of the adrenals for ascorbic acid indicated concentrations of 135 mg. per cent for the control group and 142 mg. per cent for the ACTH-treated group (Table 2).

In a second experiment a total dose of 4 mg. of ACTH was injected daily in two divided doses of 2 mgs. each for 7 days (Table 2). Results were comparable to that obtained in the first experiment in that ACTH caused a definite enlargement of the adrenal gland. The control birds possessed adrenal glands weighing 12.6 mg. and the adrenals of the treated birds weighed 18.6. Thus on an absolute weight basis, ACTH

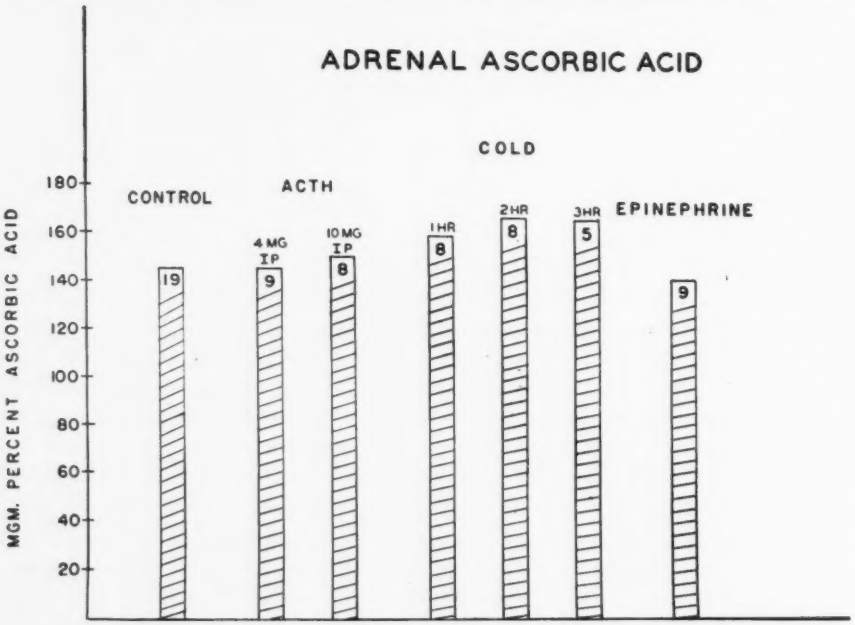

FIG. 1. The effect of ACTH, cold and epinephrine on the ascorbic acid of the adrenal gland of the quail. The number of birds used in each group is indicated at the top of each bar. Note the failure of the various stimuli to produce any significant change in the adrenal ascorbic acid concentration.

caused a 25 per cent increase in the first experiment and a 47 per cent increase in the second experiment. When the increase is determined as a function of body weight, ACTH was found to have caused a 45 per cent increase in adrenal weight in the first experiment and 71 per cent in the second experiment.

Daily injection of 100 μg. of estradiol for two weeks failed to produce hypertrophy of the adrenal gland. Implantation of 6 to 12 mg. pellets of stilboestrol however induced marked hypertrophy and hyperplasia of the gland and a slight loss in body weight. While the body weight of the stilboestrol treated birds decreased 16% the weight of the adrenal gland was approximately doubled i.e., 34.5 mg.

TABLE 2. THE EFFECT OF PROLONGED TREATMENT WITH ACTH ON ADRENAL WEIGHT AND ASCORBIC ACID

No. of quail	Treatment	Body wt., gm.			Adrenal glands		
		Start	End	Change	Wt., mg.	Body wt., mg. %	Ascorbic acid, mg. %
1st Experiment: 1 mg. ACTH daily for 14 days							
10	Controls	189	188	− 1	17.1	9.0	135
10	ACTH	181	164	−17	21.5	13.1	142
2nd Experiment: 4 mg. ACTH daily for 7 days							
10	Controls	145	153	+ 8	12.6	8.2	—
10	ACTH	138	131	− 7	18.6	14.1	—

DISCUSSION

The depletion of adrenal ascorbic acid as a result of the injection of ACTH or by such stimuli that cause the normal release of ACTH has led investigators to assume that ascorbic acid is involved in the synthesis or release of adrenal corticoids (Long 1947). Recently Jailer and Boas (1950) have shown that neither epinephrine or ACTH can alter the ascorbic acid content of the adrenal gland of the chick. This has been confirmed by Zarrow and Zarrow (1950) in the duck and the present investigation indicates that a decrease in the ascorbic acid of the quail does not accompany the release of the corticosteroids. While prolonged treatment with ACTH produced an enlargement of the adrenal gland of the quail, a single injection failed to deplete the ascorbic acid. Thus the morphogenetic action of the ACTH preparation was demonstrated in the absence of any effect on the adrenal ascorbic acid. It is also of interest to note that such stimuli as epinephrine and cold failed to affect the adrenal ascorbic acid. In the latter instance the birds were exposed to a temperature of 2 to 4° C. for a period of 1 to 3 hours and still failed to show any change in the adrenal ascorbic acid. It would seem that in the Aves at least, if one can generalize from the results on three species, there is no adrenal ascorbic acid depletion under stress as is noted in rats, guinea pigs and mice. Even in the mammals an exception has been noted in that ACTH produced little to no decrease in the ascorbic acid of the adrenal gland of the hamster (Alpert, 1950).

In spite of the failure of ACTH to deplete the adrenal ascorbic acid, chronic injection of the hormone produced significant enlargement of the adrenal gland. It is obvious then that the adrenal cortex of the bird is capable of responding to ACTH. One must conclude that a depletion of adrenal ascorbic acid is not necessary for the formation or release of adrenal corticoids or that the situation is not comparable for the various species

studied. It is of interest to note that the adrenal ascorbic acid of the quail reaches a concentration of 150 mg. per cent which compares favorably with the 200 mg. per cent noted in the chick (Jailer and Boas, 1950) and 125 mg. per cent in the duckling (Zarrow and Zarrow, 1950). This is in contrast to the more than 400 mg. per cent adrenal ascorbic acid found in the rat.

Recently a number of investigators have taken advantage of the fact that the guinea pig is unable to synthesize its own vitamin C. Long (1947) and Oesterling and Long (1951) have shown that ACTH will not affect the already depleted ascorbic acid of the scorbutic guinea pig but will cause a loss in the cholesterol concentration. Others have shown that the secretory activity of the adrenal gland is not impaired in the absence of vitamin C and have concluded that ascorbic acid is not concerned with the synthesis and secretion of adrenal corticoids (Hyman, Ragan and Turner 1950, 1951; Upton and Coon, 1951; Eisenstein and Shank, 1951). Even in the rat it has been shown that the ascorbic acid depleting potency of a preparation of the anterior pituitary is not related directly to the secretion by the adrenal gland of corticoids active on the thymus and lymph nodes (Reinhardt and Li, 1951).

It is apparent that these studies give added emphasis to the evidence indicating that ascorbic acid is not involved in the secretory activity of the adrenal. At least in the three species of birds studied thus far and in the hamster and scorbutic guinea pig a fall in adrenal ascorbic acid is not essential for the synthesis and release of the adrenal corticoids.

SUMMARY AND CONCLUSIONS

Adrenocorticotropin, epinephrine and cold failed to deplete the adrenal ascorbic acid of the quail. However, prolonged treatment with ACTH produced adrenal hypertrophy and hyperplasia as was also obtained with extremely high doses of stilboestrol.

It is concluded that the adrenal ascorbic acid concentration of quail and other birds studied is less than that found in rats and that the synthesis and release of the adrenal corticoids in quail is not dependent on a fall in the ascorbic acid of the adrenal gland.

REFERENCES

ALPERT, M.: *Endocrinology* **46**: 166. 1950.

BALDINI, J. T.: Ph.D. Thesis, Purdue Univ. Lafayette, Indiana. 1951.

EISENSTEIN, A. B. AND R. E. SHANK: 33rd Meeting, Association for the Study of Internal Secretions 69. 1951.

HYMAN, G. A., C. RAGAN AND J. C. TURNER: *Proc. Soc. Exper. Biol. & Med.* **75**: 470. 1950.

HYMAN, G. A., C. RAGAN AND J. C. TURNER: *Trans. N. Y. Acad. Sci.* **13**: 167. 1951.
JAILER, J. W. AND N. F. BOAS: *Endocrinology* **46**: 314. 1950.
LONG, C. N. H.: *Fed. Proc.* **6**: 461. 1947.
OESTERLING, M. J. AND C. N. H. LONG: *Science* **113**: 241. 1951.
REINHARDT, W. O. AND C. H. LI: *Proc. Soc. Exper. Biol. & Med.* **77**: 229. 1951.
SAYERS, G.: *Physiol. Rev.* **30**: 241. 1950.
SAYERS, G., M. A. SAYERS, E. G. FRY, A. WHITE AND C. N. H. LONG: *Yale J. Biol. & Med.* **16**: 361. 1944.
SAYERS, G., M. A. SAYERS, T. Y. LIANG AND C. N. H. LONG: *Endocrinology* **38**: 1. 1946.
SAYERS, G., M. A. SAYERS AND L. A. WOODBURY: *Endocrinology* **43**: 379. 1948.
UPTON, A. C. AND W. W. COON: *Proc. Soc. Exper. Biol. & Med.* **77**: 153. 1951.
ZARROW, M. X. AND I. G. ZARROW: *Anat. Rec.* **108**: 112. 1950.

CONSTRICTIVE RENAL HYPERTENSION IN RATS HAVING ADRENAL AUTOTRANSPLANTS WITH HEPATIC PORTAL DRAINAGE*

L. J. RATHER

Department of Pathology, Stanford University School of Medicine, San Francisco

IT IS generally conceded that the adrenal glands are necessary for the development and maintenance of renal hypertension although Rogoff Nixon and Stewart (1939) claimed that this was a non-specific effect due to the poor condition of the experimental animals after adrenalectomy and, more recently, Knowlton, Loeb, Seegal, Stoerck and Berg (1950) have reported that elevation of blood pressure in rats with nephrotoxic nephritis is not prevented by prior adrenalectomy. Even though additional salt is given, they believe that poor condition of rats following adrenalectomy is common and may account for reported failures of the blood pressure to rise following subsequent procedures on the kidneys. The elevation in blood pressure induced in rats by the feeding of large amounts of salt is not prevented by adrenalectomy (Sapirstein, Brandt and Drury, 1950). The fact that renal hypertension does not seem to be of the same nature as salt hypertension and DCA hypertension (Corcoran, Page, Masson, Taylor and Dunstan, 1951; Shorr, 1951) may have some bearing on the contradictory findings which have been reported.

Whatever the mechanism of action of the adrenal in renal hypertension, and whether the gland is involved primarily or secondarily in the pathogenesis of the condition, it must start with the liberation of something into the blood stream. Will the passage of this material through the liver, prior to its reaching the general circulation, prevent the development or in any way alter the course of hypertension induced by a subsequent operation on the kidneys? Autotransplantation of adrenal tissue to the splenic pedicle, thereby affording the regenerated cortical tissue hepatic portal drainage, was first carried out by Butcher (1948). Bernstein (1950) performed such transplants and presented evidence that the liver did not destroy the factors in the adrenal secretion necessary for the maintenance of pregnancy and spermatogenesis. Vogt (1943) had claimed that there was a reduced content or absence of cortical hormones in the right atria

Received for publication December 18, 1951.

* Accomplished under terms of public health service grant No. H-811.

of dogs and suggested that the liver might have inactivated these hormones. There is considerable evidence indicating that the liver may inactivate estrogens (Biskind and Biskind, 1946). In addition to the passage of cortical hormones through the liver prior to reaching the general circulation, the rat with an adrenal autotransplant presents another peculiarity—the absence of adrenal medullary tissue (Butcher, 1948; Ingle and Higgins, 1938). Since it has been shown that medullary hyperplasia and hypertrophy occur in constrictive renal hypertension in rats (Rather, 1951) it is necessary, in order to assess the effect on hypertension of passage of cortical hormones through the liver, to know what effect absence of the adrenal medulla in itself has on renal hypertension.

METHOD

Male albino rats of the Stanford colony weighing approximately 170 grams at the start of the experiments were used. The stock diet contained 16% protein and 0.3% NaCl.

Group I: 25 rats were subjected to adrenal autotransplantation which was carried out by removing both adrenals, discarding one, transfixing the other with a #4 silk thread

TABLE 1

Operative procedure	No. rats	Mean heart weight*
Portal adrenal transplant with subsequent removal of one kidney and handling of the other	4	102
Portal adrenal transplant with subsequent constriction of one kidney and removal of the other	11	116
Medullectomy in situ, removal of one kidney, handling of the other	4	100
Medullectomy in situ, removal of one kidney, constriction of the other	8	116

* Expressed as per cent of predicted normal value based on body weight/heart weight tables for this colony (15). The mean difference in heart weight between the portal adrenal rats with and without renal constriction is 14% and is significant ($P<0.01$). The mean difference in heart weight between the medullectomized rats with and without renal constriction is 16% and is significant ($P<0.02$).

and anchoring it in the web of mesentery at the hilus of the spleen, drawing over it a fold of mesentery and pancreas in order to reduce the possibility of the formation of adhesions. When the spleen was replaced in the abdominal cavity the transplant was located on the under surface in a somewhat protected position. The rats were then given the stock diet and 0.9% NaCl drinking water ad lib. On the 28th post-operative day the saline was withdrawn and a satisfactory weight gain ensued, suggesting adequate function of the transplant. Ingle (1938) has shown that in adrenal autotransplants the cortex and medulla become completely necrotic and growth of new cortical tissue, requiring about 30 days for completion, takes place from the capsule. The 12 surviving rats were subjected to the removal of one kidney and constriction of the other with a figure-of-eight silk ligature, according to the technique of Grollman (1944), on the 80th day after the autotransplantation.

Group II: 20 rats were subjected to adrenal autotransplantation as described above, taken off saline on the 20th day, and of the 7 survivors remaining by the 50th day 3 were subjected to the removal of one kidney and constriction of the other, while 4 were subjected to removal of one kidney with handling and replacement of the other.

Group III: 20 rats were medullectomized. The adrenals were exposed, the capsules incised and the whole adrenal expressed. The rats were given the stock diet and 0.9% NaCl for drinking water until the 23rd day, when the saline was withdrawn. They continued to gain weight in a satisfactory fashion. On the 36th day the 15 surviving rats were subjected to removal of one kidney and constriction of the other, or to removal of one kidney and handling of the other. Measurement of blood pressure: the Freidman-Freed (1949) tail microphonic manometer was used according to a procedure previously described in detail (Rather, 1950). Termination of the experiment: the 7 surviving rats of Group I were killed 141 days, the 7 rats of Group II 50 days, and the 12 rats of Group III 25 days after the renal operation. Organ weights were determined to the nearest milligram. The heart weights are analyzed in Table 1.

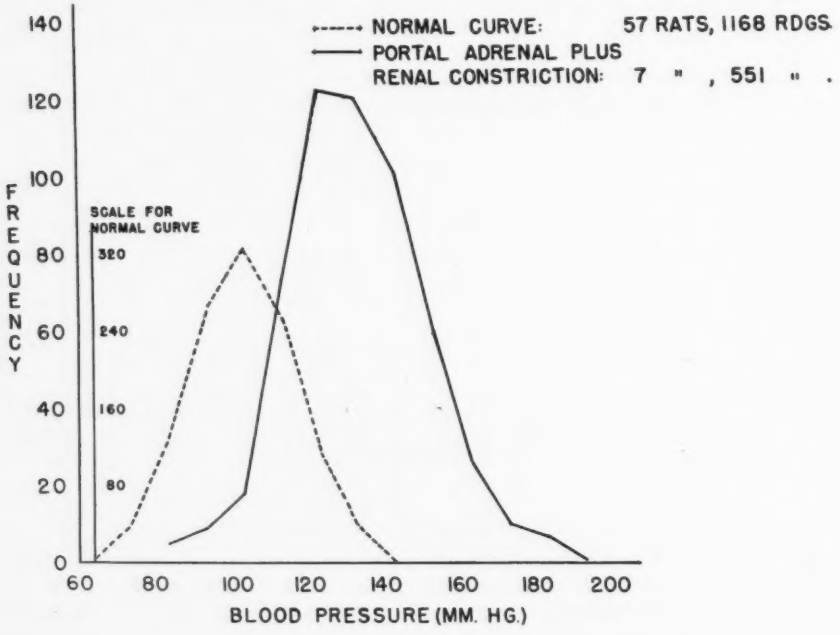

FIG. 1

RESULTS

Fig. 1 shows the frequency distribution curve of blood pressures obtained on the seven rats of Group I (subjected to unilateral nephrectomy and contralaterial renal constriction following transplantation of the adrenals to the portal area) compared with a curve based on readings from

57 normal rats of the same age. A reduced frequency scale is used for the normal curve. The mean of all readings on the normal group is 103 and that of the portal adrenal, renal constriction group is 134. The critical ratio of the differences of the means is 27. Fig. 2 shows the same normal curve plotted with two curves representing the readings of the rats of Group II. The curve of 420 readings on 7 rats with adrenals having hepatic

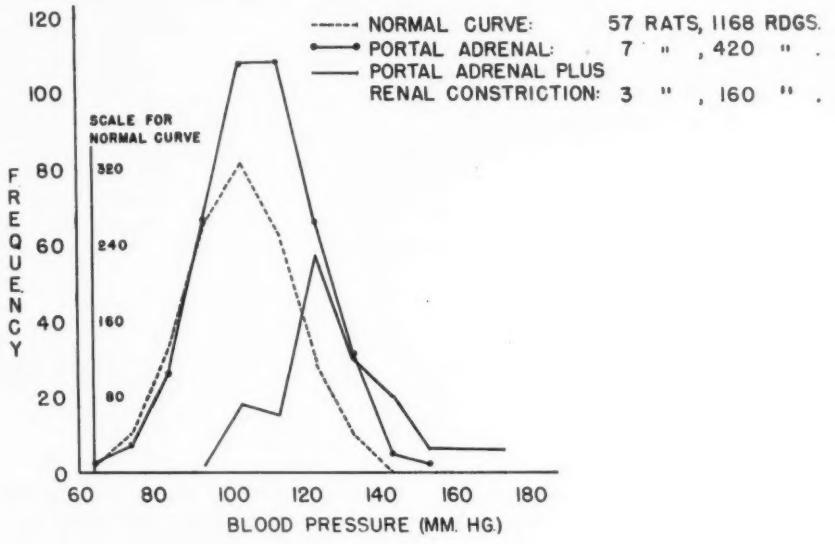

FIG. 2

portal drainage with intact kidneys does not differ significantly from that based on the readings from 57 normal rats. However, the curve representing the readings on the 3 rats subjected to unilateral nephrectomy and contralateral renal constriction following the adrenal transplantation is significantly different. The mean of this curve is 129, while that of the portal adrenal, intact kidney group is 107, the critical ratio of the difference of the means being 9.6. Figure 3 is a plot of the mean of the systolic pressures against days before and after the several operative procedures. The 7 rats subjected to adrenal transplantation are plotted as a single group prior to the renal operations. The divergence of the lines following the renal operations shows that elevation of blood pressure occurred only in the group subjected to renal constriction in addition to unilateral nephrectomy. Fig. 4 shows the frequency distribution of readings of 8 rats subjected to "medullectomy" in situ, followed by removal of one kidney and constriction of the other, compared with the curve for normal

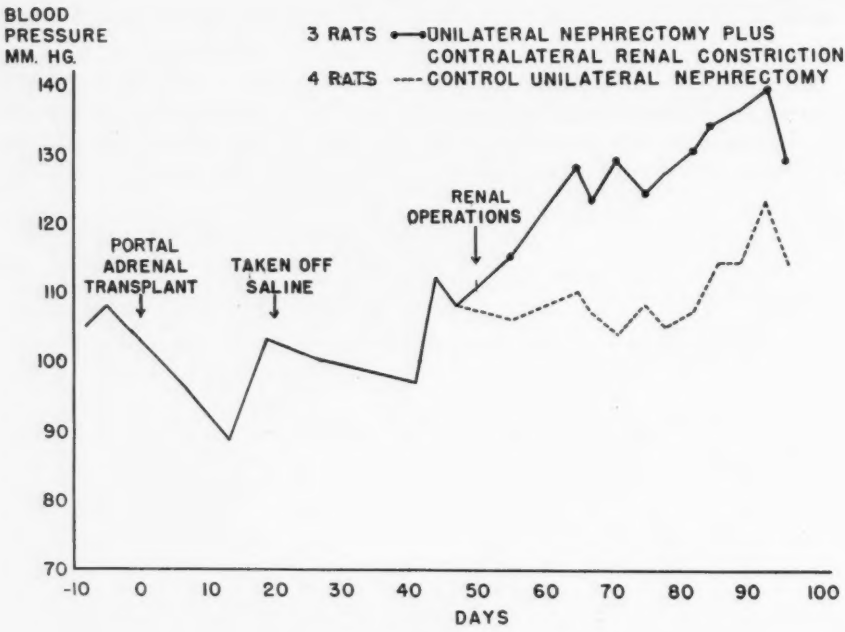
BLOOD PRESSURE MM. HG.
3 RATS —•—UNILATERAL NEPHRECTOMY PLUS CONTRALATERAL RENAL CONSTRICTION
4 RATS ---- CONTROL UNILATERAL NEPHRECTOMY
140
130
120
110
100
90
80
70
PORTAL ADRENAL TRANSPLANT
TAKEN OFF SALINE
RENAL OPERATIONS
-10 0 10 20 30 40 50 60 70 80 90 100
DAYS

FIG. 3

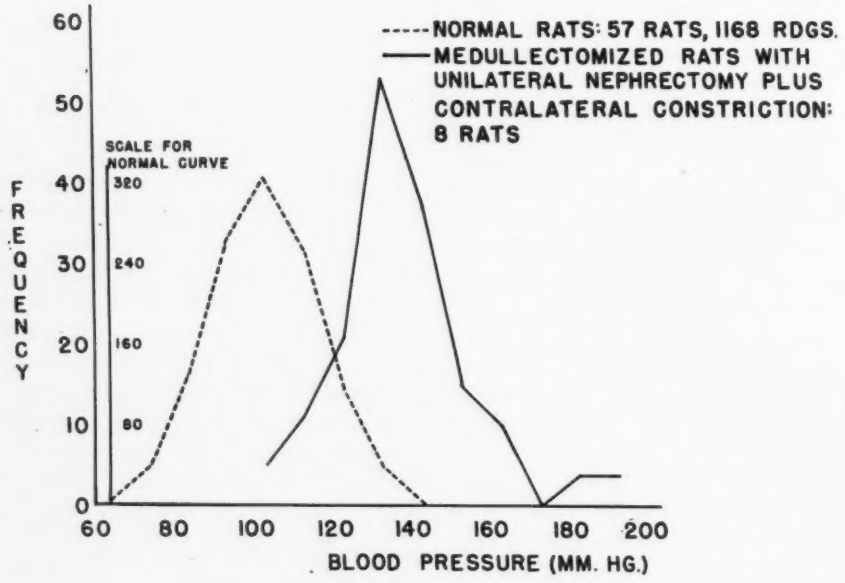
-----NORMAL RATS: 57 RATS, 1168 RDGS.
——MEDULLECTOMIZED RATS WITH UNILATERAL NEPHRECTOMY PLUS CONTRALATERAL CONSTRICTION: 8 RATS
SCALE FOR NORMAL CURVE
320
240
160
80
FREQUENCY
60
50
40
30
20
10
0
60 80 100 120 140 160 180 200
BLOOD PRESSURE (MM. HG.)

FIG. 4

rats. The mean of all of the readings on the medullectomized rats subjected to removal of one kidney and constriction of the other is 136. That of the 1168 readings on the normal rats is 104. The critical ratio of the difference of the means is 9.4. Fig. 5 shows that the 4 rats subjected to unilateral nephrectomy after medullectomy showed no elevation of blood pressure, while the 8 rats subjected to contralateral renal constriction did.

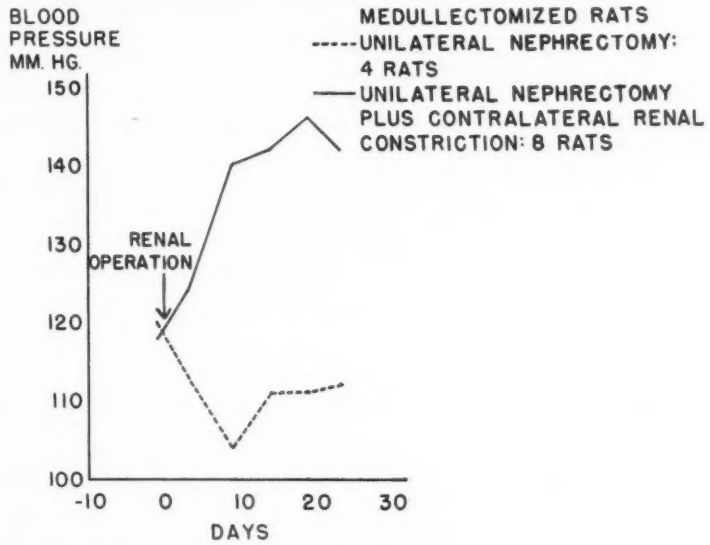

FIG. 5

HISTOLOGICAL FINDINGS

In both transplant and in situ restored adrenal tissues a distinct zona glomerulosa and zona fasciculata was present. In place of the medulla there was faintly basophilic, periodic acid-Schiff positive, debris, occasionally within multinucleated, presumably phagocytic cells.

CONCLUSIONS

Constriction of one kidney and removal of the other leads to an immediate increase of blood pressure in both "medullectomized" rats (with adrenal cortical tissue regenerated in situ) and rats with adrenal cortical transplants having hepatic portal drainage. There is no difference in the degree of elevation of blood pressure in these two groups. As compared with rats with intact adrenals subjected to the renal operations (Rather, 1950) there was less variation in the range of readings encountered. The modification in the spread of blood pressure readings obtained, in particular the absence of readings beyond 200 mmg. Hg. cannot be

attributed to an effect of the liver on adrenal hormones, since it was observed also in the "medullectomized" rats. Either it is of no particular significance or it is somehow related to the absence of the medulla. The former of these possibilities seems the most likely.

Acknowledgement

The author wishes to acknowledge the valuable technical assistance of Mrs. E. Jensen.

REFERENCES

Addis, T. and H. Gray: *Growth* **14**: 49. 1950.
Bernstein, D.: *Proc. Soc. Exp. Biol. & Med.* **75**: 847. 1950.
Biskind, G. R. and M. S. Biskind: *Am. J. Clin. Path.* **16**: 737. 1946.
Butcher, E. O.: *Endocrinology* **43**: 30. 1948.
Corcoran, A. C., I. H. Page, G. M. C. Masson, R. D. Taylor and H. Dunstan. *Arch. Int. Med.* **87**: 732. 1951.
Friedman, M. and S. C. Freed: *Proc. Soc. Exp. Biol. & Med.* **70**: 672. 1949.
Grollman, A.: *Proc. Soc. Exp. Biol. & Med.* **57**: 102. 1944.
Ingle, D. W. and G. M. Higgins: *Endocrinology* **22**: 458. 1938.
Knowlton, A. I., E. N. Loeb, B. C. Seegal, H. C. Stoerck and J. L. Berg: *Proc. Soc. Exp. Biol. & Med.* **74**: 661. 1950.
Rather, L. J.: *J. Exp. Med.* **92**: 159. 1950.
Rather, L. J.: *J. Exp. Med.* **93**: 573. 1951.
Rogoff, J. M., E. N. Nixon and G. N. Stewart: *Proc. Soc. Exp. Biol. & Med.* **41**: 57. 1939.
Sapirstein, L. A., W. Brandt, and D. R. Drury: *Fed. Proc.* **9**: 112. 1950.
Shorr, E.: Hypertension, A Symposium. Ed. by E. T. Bell (Minneapolis, Univ. of Minnesota Press. 1951).
Vogt, M.: *J. Physiol.* **102**: 341. 1943.

A SEX DIFFERENCE IN THE TOLERANCE OF WISTAR RATS FOR POTASSIUM

THEODORE ROBERTSON

From the Department of Pathology, New York Hospital, Cornell Medical Center, New York, New York

DURING the modification of a potassium tolerance test as a measure of adrenal cortical function in rats and mice (Robertson 1949) it became apparent that young female rats reacted differently to the test than did male rats of the same age. Since, in addition to the adrenal cortical hormones, the steroid hormones of the ovaries and testes have been shown experimentally to exert some influence on potassium retention and excretion (Miller 1943) the phenomenon was further explored.

MATERIALS AND METHODS

The multiple injection potassium tolerance test consists of four intraperitoneal injections of potassium chloride solution given at intervals of two hours. Rats receive a 10% solution and mice a 2% solution. The animals are weighed immediately before the test and the dose for each injection is calculated in milligrams of KCl per 10 grams of animal. Animals dying during the test or within two hours after the last injection are considered to have succumbed to the test.

Since it was necessary to rule out differences in the adrenal cortical hormone secretion of the two sexes, adrenalectomized as well as normal animals were studied. Adrenalectomies were performed under light Nembutal-ether anesthesia through bilateral posterior subcostal incisions. These animals were maintained on 1% sodium chloride drinking water and were tested on the 6th post-operative day since the work of Friedman, Mackenzie, and Friedman (1948) suggests that by this time renal function has returned to normal, insofar as possible. Adrenalectomized animals surviving the test were maintained on NaCl for one more day and then returned to plain drinking water. Of these surviving animals only those that died of adrenal insufficiency within 30 days after adrenalectomy were included in the calculations, since rats that lived longer were often found at necropsy to have persisting adrenal remnants, as others have previously noted.

Wistar rats of the Carworth Farms strain were used throughout the investigation. In about one-half of the experiments litter-mates of both sexes were tested at the same time. Inbred and crossbred mice of Ak, Rf, A, and Rockefeller strains which had been raised in our laboratory were used. They weighed from 18–40 gm.

The 50% mortality (LD50) of each group was determined by means of logarithmic-probit graph paper (Miller and Tainter 1944) and the probable errors of the LD50 of the groups and the differences between groups were calculated.

Received for publication December 19, 1951.

Presented in part before the American Society for Experimental Pathology on April 22, 1949.

EXPERIMENTAL FINDINGS

From Table 1 it will be seen that young adult female rats (2½ months old) had a greater tolerance for injected potassium than did males of the same age. This was also true of adrenalectomized rats of this age. The results as presented are a compilation of a number of experiments in which groups of male and female, adrenalectomized and intact rats of the same age were tested on the same day under similar circumstances. In each experiment the results as presented are a compilation of a number of experiments. In each experiment the results followed the pattern revealed in the compilation.

TABLE 1. EFFECTS OF MULTIPLE INJECTIONS OF POTASSIUM ON NORMAL AND ADRENALECTOMIZED RATS OF VARIOUS AGES

Potassium toxicity test			Results			
Animal groups	Age (months)	Dosage* Mgm. KCl/10 gm. rat	Rats died/rats tested			LD_{50}
			Males	Females	Total	
Normal rats	2½	3.5	0/4	—		Males: 4.00 ±0.081 Females: 4.7 ±0.103
		4.0	9/17	0/12		
		4.5	14/15	3/11		
		5.0	13/13	8/10		
	3½–8	4.0	0/4	2/5	2/9	4.12 ±0.094
		4.5	4/4	3/3	7/7	
		5.0	4/4	4/4	8/8	
		6.0	1/1	11/11	12/12	
Rats adrenalectomized 7 days prior to test	2½	1.75	2/3	—		Males: 1.65 ±0.080 Females: 2.30 ±0.095
		2.0	17/19	2/15		
		2.4	—	2/3		
		3.0	10/10	5/5		
	3½–8	1.5	0/2	—	0/2	1.80 ±0.051
		1.75	—	1/3	1/3	
		2.0	6/6	4/5	10/11	
		2.4	—	1/1	1/1	
		3.0	—	1/1	1/1	

* Given in 10% solution at each of 4 intraperitoneal injections at 2 hour intervals.
The 2½ month old rats weighed from 170–200 gm.; the 3½–8 month old rats weighed from 200–450 gm.
The Carworth Farms strain of Wistar albino rats was used.
A careful analysis of the results failed to show a significant difference in potassium tolerance among the 3½–8 month old groups.

From the findings as a whole it becomes apparent that the LD50 of intact 2½ months old male rats was 4.0 ±0.081 mg. KCl per 10 gm. body weight, while that of similar females was 4.7 ±0.103 mg. KCl. The difference here (0.7 ±0.13 mg.) is more than five times that which might occur by chance. Similarly, with adrenalectomized rats of this age group the LD50 of males was 1.65 ±0.08 mg. KCl per 10 gm. body weight and that of females was 2.30 ±0.095 mg. KCl, with a difference of 0.65

± 0.12, also five times its probable error. In each case the LD50 for normal intact rats was at least twice that for adrenalectomized rats.

With older rats ($3\frac{1}{2}$–8 months of age) no difference due to sex could be demonstrated. Neither was there a significant difference between the LD50 of the intact young adult male rats and that of the older group nor between the LD50 of the adrenalectomized young adult males and that of the adrenalectomized rats of the older group. Again in the older rats the LD50 of normal intact rats (4.12 ± 0.094) was more than twice that of similar adrenalectomized rats (1.80 ± 0.051).

Table 2 reveals that no similar increased tolerance on the part of female mice was found when a large number of intact and adrenalectomized mice of all ages were tested.

TABLE 2. EFFECTS OF MULTIPLE INJECTIONS OF POTASSIUM ON NORMAL AND ADRENALECTOMIZED MICE OF VARIOUS AGES

Potassium toxicity test		Results			
Animal groups	Dosage, Mgm. KCl/10 gm. mouse*	Mice died/mice tested			LD_{50}
		Males	Females	Total	
Normal mice	3.0	0/4	0/7	0/11	4.9 ± 0.0866
	4.0	0/18	2/20	2/38	
	5.0	11/19	8/14	19/33	
	5.5	14/17	18/18	32/35	
	6.0	7/7	10/10	17/17	
Mice adrenalectomized 7 days prior to test	2.0	3/8	3/9	6/17	2.15 ± 0.0114
	2.5	5/7	3/4	8/11	
	3.0	11/11	10/11	21/22	
	4.0	—	8/8	8/8	

* Given in 2% solution at each of four intraperitoneal injections at two hour intervals. The mice in both groups were of several breeds and weighed 18–40 gm. No variations could be related to age, sex, breed, or weight of the animals.

Studies of vaginal smears taken of the young adult female rats at the time of adrenalectomy and of sections of ovaries and uteri removed at necropsy failed to show any relation between the phase of the estrous cycle and the susceptibility to potassium.

Histological studies of the adrenals by means of Sudan stains revealed the "clear" or fat-free zone at the junction of the zona glomerulosa and the zona fasciculata that has been described by Greep and Deane (1947). This was found only in adrenals from male rats, however, and consists of large cells with a cytoplasm that is vacuolated but does not take the fat stain. Similar cells are found in the adrenals of female rats but here they are scattered indiscriminately throughout the two zones and do not form a "clear"zone. This observation can be made in adrenals from rats of all ages.

DISCUSSION

The significance of the increased tolerance to potassium manifested by young female rats is not apparent. The procedure used appears to test the ability of an animal to prevent the accumulation of a fatal concentration of potassium in the extracellular fluid when relatively large amounts of the ion are administered repeatedly. So far as is known, this may be accomplished only by excretion of the ion through the kidney or by transfer of the ion into the cells. The amorphous fraction of the adrenal cortex is said to be the hormone which is most active in the control of potassium and sodium in the body. In the experiments of this study, however, the relatively greater tolerance of young female rats as compared to young males was not affected by removal of the adrenals. If the increased tolerance of young female rats is due to their being female it would seem to be effected through some medium other than that of adrenal function.

A toxic accumulation of potassium in adrenalectomized animals is presumed to be one of the reasons why such animals die. Attempts to demonstrate a difference between male and female rats and mice in their ability to survive adrenalectomy have been largely unsuccessful. In this laboratory investigation of the survival time after adrenalectomy of intact and gonadectomized male and female rats and mice maintained on sodium chloride has failed to reveal any reproducible difference between groups. It is known that progesterone, estrogens, and testosterone can control potassium metabolism to a slight extent and that of these three hormones testosterone is the least effective (Miller 1943). Gaunt and Hays (1938) have shown that either crystalline progesterone or pseudopregnancy with its natural progesterone secretion will maintain the life of adrenalectomized ferrets. With a lack of more specific evidence it can only be assumed that the young female rats in this study had the ability, presumably due directly or indirectly to an ovarian secretion, to handle toxic doses of potassium in a more efficient fashion than did their male litter-mates. This ability has not been demonstrated previously.

The difference in the distribution of fat in the adrenal of female rats from that frequently described in male rats does not help to explain the greater potassium tolerance of young female rats. It may be, however, that these phenomona are related in that both are secondary to an undisclosed factor which operates at maximum intensity in the early mature life of the female rat.

SUMMARY

Rats of various ages were subjected to a potassium tolerance test which consists of four intraperitoneal injections of KCl solution given at

intervals of two hours. Both intact and adrenalectomized female rats $2\frac{1}{2}$ months old showed a greater tolerance for potassium than did comparable male rats of the same age; this was not so when older male and female rats were tested. Thus the LD50 of intact $2\frac{1}{2}$ months old female rats was 4.7 ± 0.103 mg. KCl per 10 gm. of body weight while that of similar males was 4.0 ± 0.081 mg. KCl. With adrenalectomized rats of this age the LD50 for females was 2.30 ± 0.095 mg. KCl per 10 gm. of body weight and that for males was 1.65 ± 0.08 mg. The differences proved statistically significant.

This phenomenon was not observed in mice studied in a similar manner.

The distribution of lipids in the adrenal cortex of female rats differs from that found in the adrenal cortex of similar male rats. The possibility is briefly discussed that an ovarian secretion in some way enables the young female rats to dispose of the excess potassium in a more efficient manner than can their male counterparts, and that this action is independent of adrenal cortical secretion.

REFERENCES

FRIEDMAN, S. M., K. R. MACKENZIE AND C. L. FRIEDMAN: *Endocrinology* **43**: 123. 1948.
GAUNT, R. AND H. W. HAYS: *Am. J. Physiol.* **124**: 767. 1938.
GREEP, R. O. AND H. W. DEANE: *Endocrinology* **40**: 417. 1947.
MILLER, H. C.: *Endocrinology* **32**: 443. 1943.
MILLER, L. C. AND M. L. TAINTER: *Proc. Soc. Exper. Med. and Biol.* **57**: 261. 1944.
ROBERTSON, T.: *Fed. Proc.* **8**: 36. 1949.

PHYSIOLOGICAL INTERACTION OF CORTISONE ACETATE AND DIETHYLSTILBESTROL. LACK OF ANDROGENIC OR ESTROGENIC EFFECTS OF CORTISONE ACETATE[1]

PAUL TALALAY,[2] MARIE MOLLOMO DOBSON, CARL M. EBERSOLE AND CHARLES HUGGINS

From the Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Illinois

IT HAS been shown that appreciable regression of human prostatic and mammary neoplasms occurs in some cases after bilateral adrenalectomy (Huggins and Bergenstal, 1951; 1952) with the use of cortisone acetate as maintenance substitution therapy. Since these tumors are neoplasms which are known to be influenced by sex hormones, it became important to know whether cortisone acetate had androgenic or estrogenic effects and if this compound could modify significantly the physiologic action of sex hormones on the accessory sex structures. These experiments will demonstrate that cortisone acetate does not stimulate the canine prostate nor the uterus of the rat but inhibits partially the action of diethylstilbestrol on the uterus.

Kochakian (1944) found that the implantation of a pellet of cortisone in castrate male mice did not cause growth of the accessory reproductive structures. The administration of cortisone acetate to intact male rats did not greatly modify the histological appearance of the testes or seminal vesicles (Winter, Silber and Stoerk, 1950; Ingle, 1950). In man cortisone acetate caused a prompt and significant decrease in the urinary excretion of 17-ketosteroids (Sprague, Power and Mason, 1950; Wilkins *et al.*, 1951) and a decrease in the signs of masculinization of girls with congenital adrenal hyperplasia, (Wilkins *et al.*, 1951).

Byrnes and Shipley (1950) observed that cortisone acetate administered to rats in daily doses of 0.5–2.0 mg. did not increase uterine weight. They also presented evidence that this compound did not inhibit gonadotrophin production by the pituitary.

Received for publication December 22, 1951.

[1] This study was aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research and from the American Cancer Society recommended by the Committee on Growth of the National Research Council.

[2] Senior Assistant Surgeon, Federal Security Agency, United States Public Health Service.

METHODS

a. Estrogenic studies. The estrogen bioassay was patterned after that described by Hertz and Tullner (1949). Twenty-four or 25 day old female Sprague-Dawley rats were ovariectomized under intraperitoneal nembutal anesthesia. Following a 7-day rest period, animals weighing between 55 and 75 gms. were divided into groups of 5–10 and injections begun on the 31st or 32nd day of life. Purified diethylstilbestrol was administered subcutaneously in aqueous solutions containing 0.5 per cent Tween 40 (as a suspending

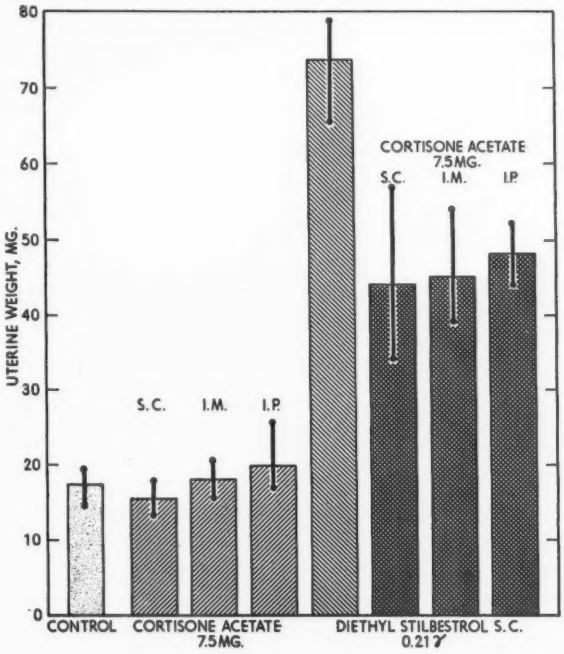

FIG. 1. Uterine weights of rats injected with cortisone acetate, diethylstilbestrol, or combinations. The cortisone was injected in 3 sites, subcutaneously, intramuscularly or intraperitoneally. The vertical lines represent the magnitude of the standard deviations of the mean uterine weights.

agent). Total doses of 0.15–0.30γ per animal were given in 3 injections of 0.5 ml. each on successive days (9:00 A.M.) and the animals were sacrificed on the 4th morning, approximately 24 hours after the last estrogen injection. Control animals received equivalent volumes of the injection medium. At sacrifice, the weight of the body of the animals was determined, the uteri dissected out, blotted on filter paper and weighed on a torsion balance to the nearest 0.2 mg.

Cortisone acetate (Cortone, Merck) was appropriately diluted with saline and injections were given by indicated route, twice daily about 30–60 minutes prior to the morning estrogen injection and in the afternoon of each day. Thus there were six injections of 0.5 ml. each. Control animals received equivalent volumes and concentrations of carboxymethylcellulose. Estrogen and cortisone were given at different sites of injection.

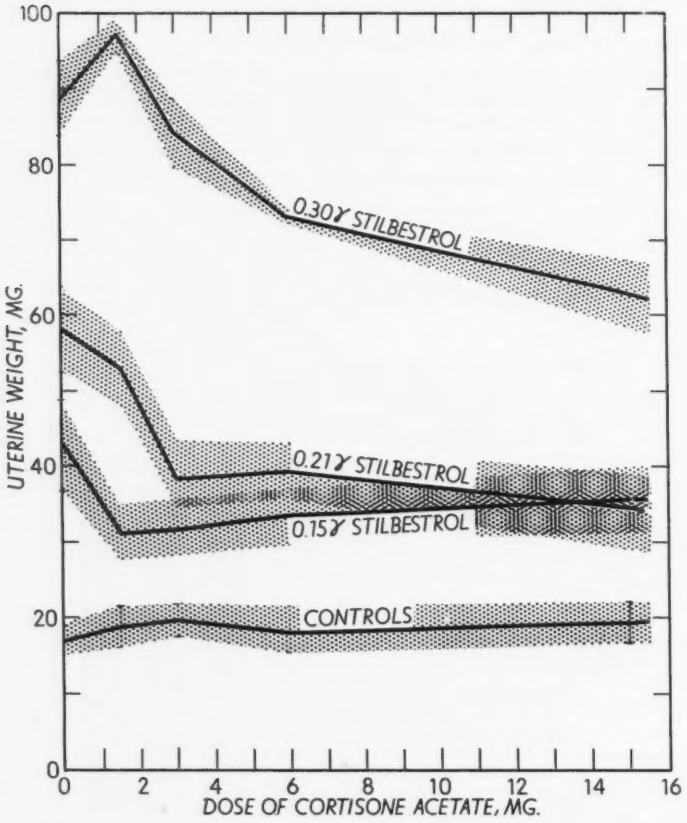

FIG. 2. Uterine weights of rats injected with cortisone acetate and diethylstilbestrol. The shaded areas represent the standard deviations of the mean uterine weights.

b. Androgenic studies. The prostatic secretions of 4 normal adult dogs were collected for many days before, during, and after the administration of cortisone acetate. We employed the prostatic isolation operation (Huggins, Masina, Eichelberger and Wharton, 1939) whereby the prostate is isolated from the bladder and the urine is deviated through a cannula; a circumcision is also made. A large aluminum cannula 10 cm. long with an inside diameter of 1.2 cm. was inserted in the bladder and brought out through a "stab" incision in the left lower quadrant of the abdomen. These procedures permit the quantitative collection of prostatic secretion after healing is complete. The prostatic fluid was collected every 2 days for 1 hour following the intravenous injection of pilocarpine hydrochloride, 5 mg. in 1 ml. of saline. Cortisone acetate was injected intramuscularly each day in 2 divided doses, in total daily amounts of 25 mg., and later of 50 mg. for periods of 14 to 24 days in each case.

RESULTS

a. Estrogenic studies. Figure 1 shows a typical experiment in which control uterine weight averaged 17.5 (±2.4) mg.; 0.21 γ diethylstilbestrol stimulated the uterine weight to 73.7 (±4.7) mg. A total dose of 7.5 mg. cortisone given over a 3-day period by intramuscular, subcutaneous or intraperitoneal routes produced no significant change in uterine weight as compared to the controls. When administered with estrogen, 7.5 mg. cortisone reduced the estrogenic stimulation of 0.21 γ stilbestrol from 73.6 mg. to an average of 46 mg.

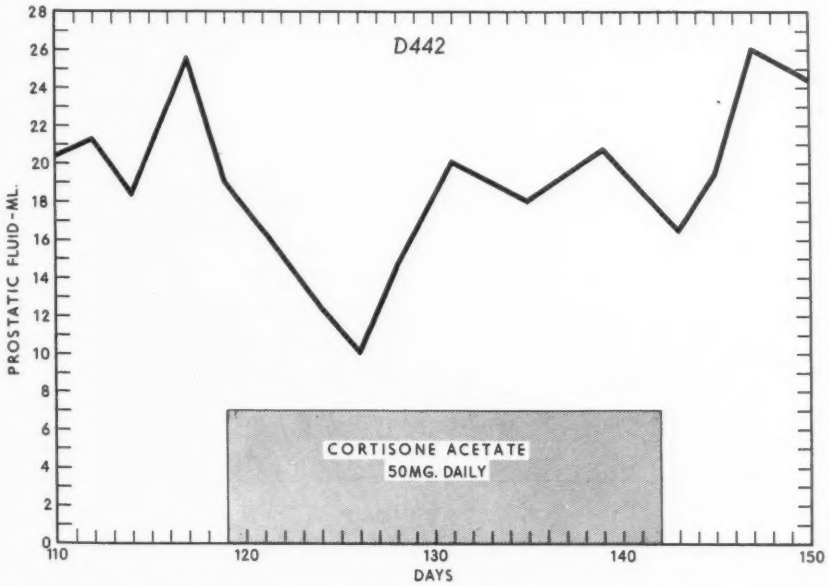

FIG. 3. Excretion of prostatic fluid by a normal dog injected with cortisone acetate.

Figure 2 shows the interaction of varying doses of cortisone and of estrogen. Cortisone doses of 1.5–15 mg. were without effect on the uterine weight of the control rats although the larger doses produced some loss of body weight. Complete suppression of estrogenic stimulation by cortisone in even relatively large doses was not observed. Within limits there appears to exist a competitive type of interaction between cortisone and stilbestrol, larger doses of one requiring larger doses of the other for partial neutralization.

b. Androgenic studies. Soon after beginning the injections of cortisone

acetate there was a slight loss of body weight (average maximum decrease 0.6 kg.) but in each instance body weight was restored to the pre-treatment level before the 10th day of administration of the compound.

In all of the dogs there was a slight decrease in the secretion of prostatic fluid (Fig. 3) during the administration of cortisone acetate but there was a later recovery to the pre-treatment levels while the compound was being given.

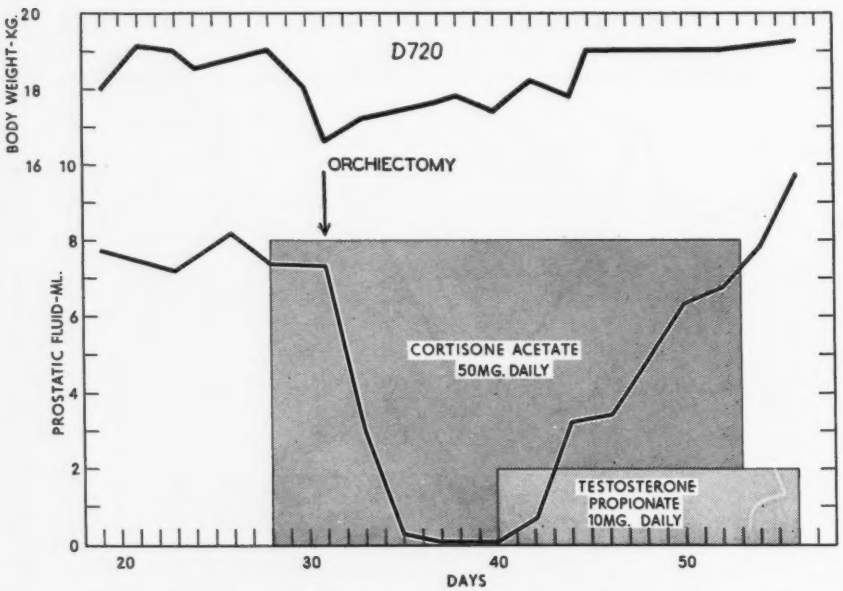

FIG. 4. Excretion of prostatic fluid by a normal dog. Despite the administration of cortisone acetate, prostatic secretion was abolished by orchidectomy and restored by the subsequent administration of testosterone propionate.

When orchiectomy was done 2 days after commencing injections of cortisone acetate the secretion of prostatic fluid ceased (Fig. 4). At the time when there was no prostatic secretion, intramuscular injections of testosterone propionate, 10 mg. daily, were begun, continuing cortisone acetate, 50 mg. daily. The prostatic secretion was restored at a normal rate (Huggins, 1946; 1947). These observations indicate that cortisone acetate does not maintain the prostatic epithelium at a functioning level after orchiectomy and is proof that cortisone acetate does not block the androgenic activity of testosterone propionate at the stated dose levels.

CONCLUSIONS

Cortisone acetate is neither androgenic nor is it an antagonist to testosterone propionate with respect the prostatic epithelium of the dog. We were able to confirm earlier observations that cortisone acetate is without effect on the weight of the uterus of the castrate immature female rat. When given in conjunction with diethylstilbestrol, cortisone acetate partially suppresses the stimulating effect of this estrogen on the uterus in what appears to be a competitive manner.

REFERENCES

Byrnes, W. W. and E. G. Shipley: *Proc. Soc. Exp. Biol. & Med.* **74**: 308. 1950.
Hertz, R. and W. W. Tullner: *Endocrinology* **44**: 278. 1949.
Huggins, C., Harvey Lectures **42**: 148. 1946–47.
Huggins, C. and D. M. Bergenstal: *J. Am. Med. Assoc.* **147**: 101. 1951.
Huggins, C. and D. M. Bergenstal: *Cancer Research* **12**: 134. 1952.
Huggins, C., M. H. Masina, L. Eichelberger and J. D. Wharton: *J. Exp. Med.* **70**: 543. 1939.
Ingle, D. J.: *J. Clin. Endocrinol.* **10**: 1312. 1950.
Kochakian, C. D. *Am. J. Physiol.* **142**: 315. 1944.
Sprague, R. G., M. H. Power and H. L. Mason: *J. Am. Med. Assoc.* **144**: 1341. 1950.
Wilkins, L., R. A. Lewis, R. Klein, L. I. Gardner, J. F. Crigler, Jr., E. Rosemberger and C. J. Migeon: *J. Clin. Endocrinol.* **11**: 1. 1951.
Winter, C. A., R. H. Silber and H. C. Stoerk: *Endocrinology* **47**: 60. 1950.

NOTES AND COMMENTS

THE THERMOGENIC EFFECT OF PROGESTERONE AND ITS RELATION TO THYROID FUNCTION

The physiologic mechanisms through which progesterone exercises its thermogenic effect have received little attention. The presence of the ovaries, adrenals and thyroids have been shown to be non-essential, although a smaller quantitative effect is present in thyroidectomized rats (Nieburg and Greenblatt, 1948). The possibility that an increased secretion of thyroid hormone is responsible for the temperature rise during the ovulatory and postovulatory phases of the human menstrual cycle is suggested by Watzka (1950) on the basis of a concommitant rise, at ovulation, in body temperature, red blood cell and reticulocyte count. The latter effect, according to evidence cited by Watzka (Landsberg, 1927; Moldavsky, 1930; Mansfeld and Sos, 1938; Heilmeyer, 1942) is probably brought about by thyroid hormone. Watzka also cites his own work as evidence for a direct correlation between changes in the BMR and body temperature, and concludes that since body temperature, red blood cell count, and probably also, the BMR, increase simultaneously at ovulation, an increased secretion of thyroid hormone may be presumed responsible for all three phenomena. Other bits of evidence point to an increased secretion of thyroid hormone during the ovulatory and possibly postovulatory phases of the menstrual cycle (Conklin and McClendon, 1930; King, 1914; Hitchcock and Wardwell, 1929; Rubenstein, 1938; Kleitman and Ramsaroop, 1948). The thermogenic effect of thyroid hormone has also received some support (Maqsood, 1951). Progesterone might thus act through the thyroid hormone to effect a rise in body temperature. This possibility has been investigated as described in the present report.

EXPERIMENTAL METHODS AND RESULTS

Two experiments have been carried out. It was first postulated that if the thyroid were indeed implicated in the mediation of the thermogenic effect of progesterone, it should be difficult, or impossible, to induce a body temperature rise with progesterone in cretins. Four male cretins at the Columbus State School on whom body temperatures were being recorded as described elsewhere (Rothchild and Barnes, 1952) were injected intramuscularly with 10.0 mgs. of progesterone per day for 11 days. The mean body temperatures during the pre-progesterone, progesterone and post-progesterone periods (Figure 1) clearly show a typical body temperature elevation induced by progesterone, and a return to the control level after the cessation of progesterone administration.

The second experiment was designed to determine whether relatively high doses of progesterone would effect a change in the level of circulating thyroid hormone concommitant with the rise in body temperature. Ten high-grade feeble-minded adolescent males were injected intramuscularly daily for nine days with either 25.0 mgs. (five subjects) or 50.0 mgs. (five subjects) of progesterone. In one group blood was drawn two days, and in the other group one day before the first progesterone injection, and continued on alternate days for each boy until the fifth or seventh day after the last injection of progesterone. The bloods were allowed to clot spontaneously, and on the day after collection were assayed for protein bound blood iodine (PBI) by the method of Connor *et al.* (1949). A comparison of the changes in body temperature and the PBI, before, during, and after the progesterone injections (Figure 2) shows no correlation between the two.

Received for publication March 6, 1952.

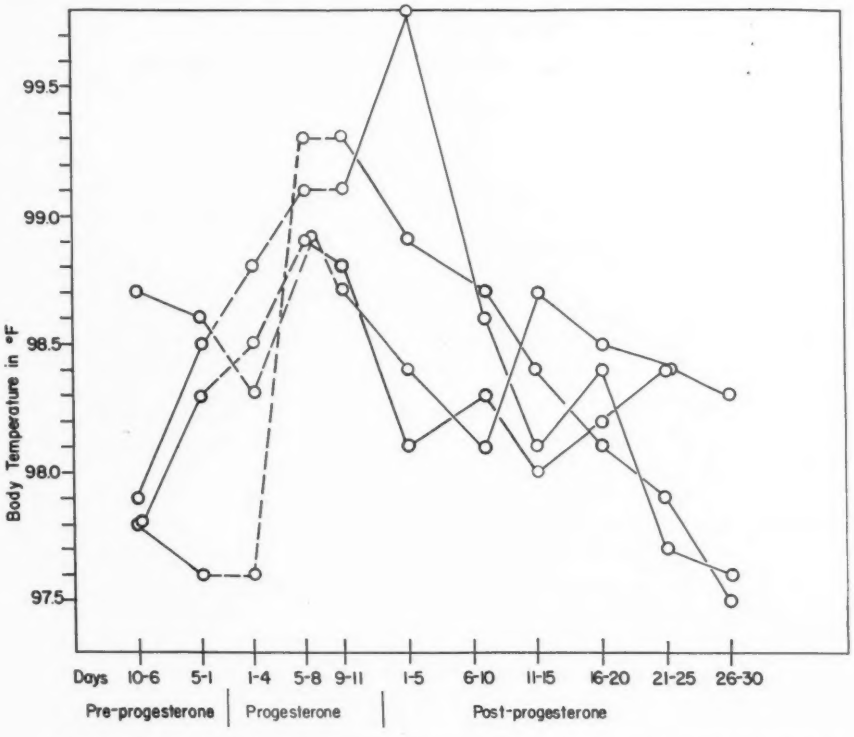

FIG. 1. *The body temperature responses of four male cretins to injections of 10.0 mgs. of progesterone per day for 11 days.* The body temperatures of each subject are shown as the means for periods of from three to five days. The portions of each curve in dotted lines represent the temperatures during the period of progesterone treatment. For discussion see the text.

DISCUSSION

These two experiments indicate that the rise in body temperature induced by progesterone is not mediated through the thyroid hormone and that no apparent change in thyroid function occurs under the influence of progesterone. Increased thyroid function may yet occur concommitantly with ovulation, even through evidence for this is meager and indirect. The increased thyroid function in early pregnancy (Peters, Mann and Heinemann, 1948) may thus reflect a level of activity continued from the luteal phase of the cycle in which conception occurred. However, from the data we have presented here, the body temperature rise associated with the luteal phase of the cycle is not dependent upon this presumed increase in thyroid activity, even if the thyroid hormone did have a thermogenic effect, and convincing evidence of such an effect is lacking. It is likely, therefore, that progesterone itself is the hormone responsible for the thermal shift of the menstrual cycle. Some discussion of the possibility that this effect may be mediated through the heat regulating centers of the hypothalmus is offered in a recent paper (Rothchild and Barnes, 1952).

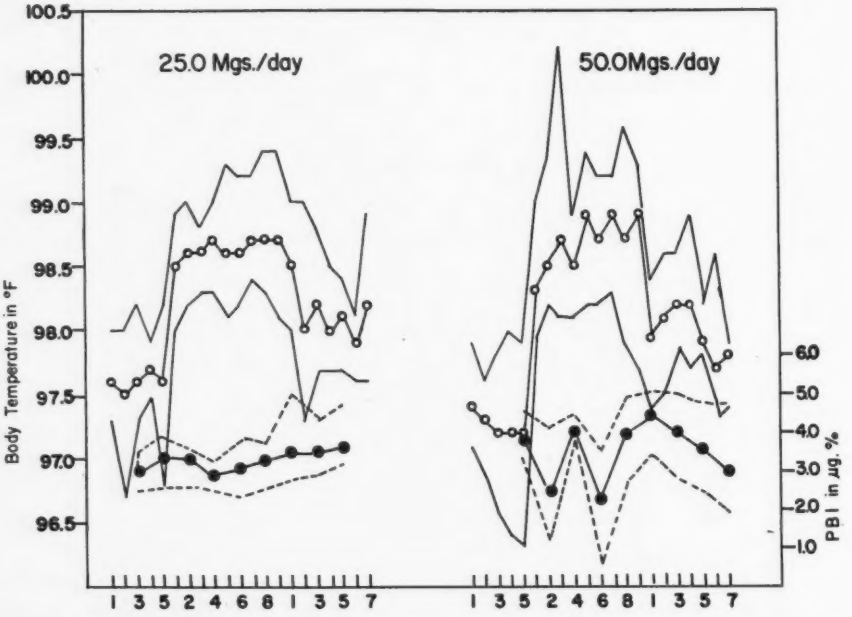

FIG. 2. *The body temperature and PBI responses to progesterone in 10 adolescent male feeble-minded subjects.* The body temperatures and PBI's are shown as the mean of the entire group (five subjects at each dose level), with the upper and lower limits of variation for each day of observation. The open circles represent the body temperature, the closed circles, the PBI. The numbers along the abscissa represent the days of observation; the first five days are the pre-progesterone control period, the next nine days are the days of progesterone treatment; the last seven days are the post-progesterone control period. The dose of progesterone administered is shown above each curve.

SUMMARY

A lack of thyroid mediation of, or intervention in the physiologic processes by which progesterone exerts its thermogenic effect has been shown by two kinds of evidence. Four cretins responded to daily injections of 10.0 mgs. of progesterone with typical body temperature elevations, and ten high-grade feeble-minded adolescent males, although showing typical body temperature elevations during a course of progesterone treatment, showed no concomitant changes in the level of circulating thyroid hormone.

REFERENCES

CONKLIN, C. J. AND J. F. MCCLENDON: *Arch. Int. Med.* **45**: 145. 1930.

CONNOR, A. C., R. E. SWENSON, C. W. PARK, E. C. GANGLOFF, R. LIEBERMAN AND G. M. CURTIS: *Surgery* **85**: 510. 1949.

HEILMEYER, L.: *Blutkrankheiten*, Springer Verlag, Berlin. 1942.

HITCHCOCK, F. A. AND F. R. WARDWELL: *J. Nutr.* **2**: 203. 1929.
KING, J. L.: *Am. J. Physiol.* **34**: 203. 1914.
KLEITMAN, N. AND A. RAMSAROOP: *Endocrinology* **43**: 1. 1948.
LANDSBERG, M.: *Med. Klin.* **23**: 1817. 1927.
MANSFELD, G. AND J. SOS: *Klin. Wchnschr.* **17**: 386. 1938.
MAQSOOD, M.: *Nature, London* **167**: 356. 1951.
MOLDAVSKY, Y. V.: *Ztschr. f. klin. Med.* **114**: 346. 1930.
NIEBURGS, H. E. AND R. B. GREENBLATT: *J. Clin. Endocrinol.* **8**: 622. 1948.
PETERS, J. P., E. B. MANN AND M. HEINEMAN: *Yale J. Biol. and Med.* **20**: 449. 1948.
ROTHCHILD, I. AND A. C. BARNES: *Endocrinology* **50**: 485. 1952.
RUBENSTEIN, B. B.: *Endocrinology* **22**: 41. 1938.
WATZKA, M.: *Deut. med. Wchnschr.* **75**: 1231. 1950.

IRVING ROTHCHILD AND RICHARD L. RAPPORT

The Departments of Gynecology and Obstetrics,
and Surgical Research
The Ohio State University College of Medicine
Columbus, Ohio

A SIMPLE METHOD FOR THE PREPARATION OF AQUEOUS SOLUTIONS OF STEROID HORMONES FOR INTRAVENOUS ADMINISTRATION

Techniques for administering steroids to human subjects by the intravenous (IV) route are far from satisfactory, or practical. Sodium estrone sulfate is the only naturally occurring steroid whose IV administration presents no special problem. While propylene glycol has been used as a steroid solvent for human use (Loeser, 1948; Wolfson, 1951) its hemolysing effects limit its application to small volumes or single injections. The "association colloids" are excellent steroid solvents (Ekwall and Sjöblom, 1950) but reports on their use for human IV administration have not appeared, and their toxicity would be, at least, subject to question. A water soluble testosterone has been prepared (sodium testosterone sulfate) (Holden *et al.*, 1949), but its biologic activity is definitely different from that of testosterone (Holden, *et al.*, 1950). Bischoff and Pilhorn (1948) described the relatively high solubility of various steroids in serum albumin solutions, and recently West *et al.* (1951, a, b, c) have used a modification of Bischoff and Pilhorn's technique for the preparation and use of IV solutions of testosterone in laboratory animals and human subjects. The sterilized crystals are shaken overnight at 37° C. with human serum albumin solution; the solution is then filtered, and chemically assayed for its hormone content (West, 1951a).

I have found recently that aqueous solutions of steroid hormones may be prepared with concentrations almost as high as those achieved by West (1951a) by a technique based on the simple combination of two of the facts mentioned in the preceding paragraph. One is that propylene glycol (PG) is a good steroid solvent, the other is that a serum albumin solution is almost equally as good. Thus, when a PG-steroid solution is added to one of human serum albumin (HSA)[1] the steroid remains dissolved in the resulting mixture. Above a critical PG/HSA ratio, protein coagulation occurs, but at any ratio below it, no apparent change in the nature of the protein is seen. For concen-

Received for publication April 10, 1952.

trated HSA solutions, (25%), this ratio is about 2.0. Above another critical PG/HSA ratio, precipitation of the steroid occurs, but at any ratio below it, a stable solution, that may be diluted as much as 100 times or more with water, is obtained. This second ratio varies with the steroid and its concentration in PG, as well as with the concentration of the HSA, and is in the process of being fully explored. The advantages of the technique over those previously described are as follows.

The steroid concentration in the final solution is determined solely by the factors of weighing and dilution, and the necessity for chemical determination is eliminated. Sterilization and asepsis are not particularly important problems, since the already sterilized HSA solutions (as well as the sterile diluting solution) is mixed with the autoclaved PG solution without violation of the rubber seals except by means of sterile needle puncture. Solution is practically instantaneous after mixture of the two solvents, and allows one therefore to prepare solutions for IV use at a moments notice. Since the solutions may be diluted with water, (isotonic saline, 10% and 5% dextrose, etc., are included in the general term "water"), non-toxic concentrations of PG can be easily prepared. The ingredients used are all relatively cheap, available and can be stored indefinitely under ordinary laboratory or hospital conditions. The solutions are admirably adaptable to either single injections or venoclysis; for example, 100.0 mgs of testosterone may be given as a single IV injection in a minimum of 50.0 cc. of 5% PG and 95% concentration HSA, or by venoclysis in a volume of any desired size above 100 cc., and in almost any desired osmotic relation to body fluids. The maximum steroid concentration is about 2.0 mgs. per cc. for testosterone, methyl testosterone, and progesterone, and about 1.0 mgs. per cc. for estradiol,[1] thus permitting administration of doses consistent with those known to be effective by other routes, without necessitating use of excessive fluid volumes. Since the free steroids may be dissolved this way, there is no change in biologic activity, except that due to the difference in route of administration. The fact that the 4 steroids mentioned show very similar solubility characteristics indicates that the method is probably adaptable, with slight modifications, to all steroid hormones. As already mentioned, this is being studied, and will be reported at a later date.

The IV route of administration permits one to overcome some of the deficiencies inherent in the other parenteral, as well as oral, routes. The technique described here, therefore, because of its simplicity, adaptability, and elimination of the use of possibly toxic substances to achieve solution of the steroids, makes it possible and practical to reinvestigate the field of steroid hormone physiology and therapy, employing the intravenous rather than the subcutaneous or intramuscular routes of administration.

IRVING ROTHCHILD

From the Departments of Obstetrics and Gynecology and of Physiology,
Ohio State University,
Columbus, Ohio

REFERENCES

BISCHOFF, F. AND H. R. PILHORN: *J. Biol. Chem.* **175:** 663. 1948.
EKWALL, P. AND L. SJÖBLOM: *Acta Endocr.* **4:** 179. 1950.
HOLDEN, G. W., I. LEVI AND R. J. BROMLEY: *J. Am. Chem. Soc.* **71:** 3844. 1949.
HOLDEN, G. W. AND E. LOZINSKI: *Endocrinology* **47:** 305. 1950.

[1] My thanks are due the American Red Cross for the supplies of concentrated human serum albumin, and Dr. Edward Henderson, of the Schering Corporation, Bloomfield, N. J., for the supplies of crystalline steroids.

LOESER, A. A.: *J. Ob. and Gyn. Br. Emp.* **55**: 17. 1948.
WEST, C. D.: *Endocrinology* **49**: 467. 1951a.
WEST, C. D., F. H. TYLER AND H. J. BROWN: *J. Clin. Endocrinol.* **11**: 883. 1951b.
WEST, C. D., F. H. TYLER, H. BROWN AND L. T. SAMUELS: *J. Clin. Endocrinol.* **11**: 897. 1951c.
WOLFSON, W. Q.: (Personal communication, 1951).

THE EFFECT OF NOR-EPINEPHRINE* ON THE EOSINOPHIL COUNT IN THE MOUSE

Recently attempts have been made to show whether nor-epinephrine acts as does epinephrine in reducing the number of circulating eosinophils. Pellegrino *et al.* (1950) and Madison (1950) concluded from their studies that it did not, while Humphreys and Raab (1950) found that it did. The three groups worked with human subjects. We have used mice as subjects and were able to confirm Humphreys and Raab.

The methods of Speirs and Meyer (1951) were employed except that animals whose adrenals had been previously enucleated were used. The results with nor-epinephrine are shown in the table.

EFFECTS OF NOR-EPINEPHRINE ON CIRCULATING EOSINOPHILS OF MICE WITH ENUCLEATED ADRENALS

Determinations	Dose gamma	Eosinophil count		Per cent change
		0 hr.	4 hr.	
24	60	52	12	−77%
8	50	48	20	−58%
8	40	41	25	−40%
10	30	50	35	−30%
10	20	62	50	−19%
6	10	55	52	−6%

It will be noted that the maximum response was obtained with 60 gamma while smaller doses produced less and less effect as they were decreased. Twenty determinations were made with 10 gamma of epinephrine, which produced the maximum effect. At the end of the test period the eosinophil count had dropped 88 per cent. Therefore nor-epinephrine was about one sixth as potent as epinephrine in reducing the eosinophil count. Apparently Pellegrino *et al.* and Madison failed to obtain an effect with nor-epinephrine because their dosage was too small.

CONCLUSION

Nor-epinephrine in doses six times that of epinephrine produces a maximum eosinopenia in mice with enucleated adrenals.

ACKNOWLEDGMENT

The author wishes to thank Dr. F. A. Hartman for his counsel in this study.

J. KIMBALL SCOTT

Dept. of Physiology,
The Ohio State University,
Columbus, Ohio

Received for publication February 4, 1952.

* We are indebted to Dr. M. L. Tainter of the Sterling-Winthrop Research Laboratories for the nor-epinephrine.

REFERENCES

HUMPHREYS, R. J. AND W. RAAB: *Proc. Exp. Biol. & Med.* **74**: 302. 1950.
MADISON, L. L.: *Clin. Investigation* **29**: 789. 1950.
PELLEGRINO, P. C., G. M. MORRIS AND S. TRUBOWITZ: *Proc. Exp. Biol. & Med.* **74**: 330. 1950.
SPEIRS, R. S. AND R. K. MEYER: *Endocrinology* **48**: 316. 1951.